Fundamentals of Biochemistry

A Textbook

H.P. Gajera
S.V. Patel
B.A. Golakiya

VISIT…

LANZAROTE
Caliente.com

Fundamentals of Biochemistry

A Textbook

H. P. GAJERA
Assistant Professor
Department of Biochemistry
College of Agriculture
Junagadh Agricultural University
Junagadh, Gujarat, India - 362 001

S. V. PATEL
Associate Research Scientist
Department of Biochemistry
College of Agriculture
Junagadh Agricultural University
Junagadh, Gujarat, India - 362 001

B. A. GOLAKIYA
Professor & Head
Department of Biochemistry
College of Agriculture
Junagadh Agricultural University
Junagadh, Gujarat, India - 362 001

International Book Distributing Co.
(Publishing Division)

Published by

INTERNATIONAL BOOK DISTRIBUTING CO.
(Publishing Division)
Khushnuma Complex Basement
7, Meerabai Marg (Behind Jawahar Bhawan)
Lucknow 226 001 U.P. (INDIA)
Tel. : 91-522-2209542, 2209543, 2209544, 2209545
Fax : 0522-4045308
E-Mail : ibdco@airtelmail.in

First Edition 2008

ISBN : 978-81-8189-165-5

© Publisher
All Rights Reserved

No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior written permission of the publisher.

Composed & Designed at :

Panacea Computers
2nd Floor, Agarwal Sabha Bhawan, Subhash Mohal
Sadar Cantt., Lucknow-226 002
Phone : 0522-2483312, 9335927082, 9452295008
E-mail : prasgupt@rediffmail.com

Printed at:

Salasar Imaging Systems
C-7/5, Lawrence Road Industrial Area
Delhi - 110 035
Tel. : 011-27185653, 9810064311

Dr. B. K. Kikani
Vice-chancellor

Junagadh Agricultural University
Junagadh- 362 001

FOREWORD

Biochemistry is a gateway of all the branches of life science. It's a field of enormous interest and utility. Biochemistry is a study of the molecule of life. Our understanding of the molecular nature of life is growing at an incredible rate. It is difficult to embody all the information related to this subject in a single collection. If at all it has been done than the user will be discouraged by its volume. It is than even more tough task to encapsulate huge bulk of literature in a small handy book. This effort is done by our team in the Department of Biochemistry, Junagadh Agricultural University, Junagadh, Gujarat, India.

The tools of biochemistry have been used to explain biological processes such as origin of life, cell development, cell differentiation, metabolisms, energy dynamics, origin and cause of diseases and even human behaviors. The principles of biochemistry are now reaching into chemistry, the health sciences, nutrition, agriculture, physiology, immunology, neurology, cell biology, biotechnology, nanotechnology, ecology, computer science and psychology. Not only the biochemistry expanding; other disciplines are using the tools of biochemistry to solve their unique problem. Thus biochemistry is seated at the core of other branches of science.

This particular branch is engaged in understanding the nature and properties of the life throbbing around us. It is at the threshold where non living biomolecules through some intricate forces starts dancing while getting alive; and we call it a living being. This science has yet to fill up a knowledge gap about secret of life itself.

This text book is a distillation of the years of experiences of our faculty members affiliated with teaching and research. Hopefully a complete grasp of the principle of biochemistry can be obtained by simply reading this book. This collection entitled **FUNDAMENTALS OF BIOCHEMISTRY: A TEXTBOOK** will cater the need of a large segment of people who have a scientific bend.

Date: 20/06/2007

Place: Junagadh

(B.K.Kikani)

“This Page is Intentionally Left Blank”

Dr. D. B. Kuchhadiya
Director of Research and
Dean P.G. Studies

Junagadh Agricultural University
Junagadh- 362 001

PREFACE

As such the subject of Biochemistry is vast in its content. Its a tough task to pack-up it in a single volume. While preparing a brief note of this subject our faculty members worked for more than 12 hours in a day behind each and every chapter even at final stage. They summarized the content without loosing the essense of the concept. I found it beautifully narrated with 145 figures & photographs, 30 tables containing total 322 text pages.

While reading any subject our mind do summarize its major points. This book has been able to develop as a ready reckoner. Its my pleasure that our faculty mambers are mastering the art of scientific writing. There is vacume in the area of lucid and lucrative scientific documentation. If we want to propagate the scientific knowledge we should present it in a more simple form, in a pictorial and diagrammatic manner. It should be more graphical and photographical. I think our team working at the Biochemistry and Biotechnology Department of Junagadh Agricultural University has to some extent fulfilled the above contention while preparing this book entitled "FUNDAMENTALS OF BIOCHEMISTRY: A TEXTBOOK".

I have no doubt that this collection will be appreciated by the students, teachers, researchers and professionals dealing with biosciences.

Date: 20/06/2007

Place: Junagadh (D.B.Kuchhadiya)

“This Page is Intentionally Left Blank”

CONTENTS

Sr. No Topics Page No

1. **Introduction** **1**
    - Origin of Life 1
    - The Elements of Life 3
    - Biomolecules to Cell 5
    - Cells and Organells 7
    - Water 16
    - History of Biochemistry 20

2. **Carbohydrates** **43**
    - Nature and Nomenclature 43
    - Classification of Carbohydrates 44
    - Monosaccharides 44
    - Oligosaccharides 54
    - Storage Polysachharides 58
    - Structural Polysachharides 61
    - Chemical Properties of Carbohydrates 67

3. **Lipids and membranes** **77**
    - Classification of Lipids 77
    - Fatty Acids 78
    - Simple Lipids 80
    - Compound Lipids 82
    - Properties of Fat 88
    - Constants of Fats and Oils 89
    - Biological Membranes 91

4. **Nucleic acids** **113**
    - Nucleic Acids Building Blocks 113
    - Nucleotide Composition 115
    - Types and Functions of Nucleic Acids 115
    - Primary Structure of Nucleic Acids 115
    - Secondary Structure of Nucleic Acids 116
    - Complementarity 118
    - DNA and Inheritance 122

5. **Amino acids and proteins** **139**
    - Chemistry of Amino Acids 139
    - Classification of Amino Acids 145
    - Essential Amino Acids 146

Properties of Amino Acids 146
Peptides 148
Classification of Proteins 148
Conformation of Proteins 154
Physical and Chemical Properties of Proteins 161
Myoglobin and Hemoglobin 162
Allosterism and Regulation 166

6. **Enzymes and Kinetics** **181**
Characteristics of Enzymes 181
Classification of Enzymes 182
Cofactor 184
Mechanism of Enzyme Action 185
Enzyme Kinetics and Inhibitors 187
Isoenzymes 196
Enzyme Regulation 196

7. **Vitamins and Co-enzymes** **225**
Fat soluble Vitamins 225
Water Soluble Vitamins 226
Co-enzymes 227

8. **Introduction to Metabolism** **257**
Metabolism Pathways 257
Characteristics of Path ways 257
Organic Reaction in Metabolisms 258
Thermodynamicas in Metabolism 259

9. **Carbohydrate Metabolism** **275**
Glycolysis 276
Gluconeogenesis 292
Pyruvate Metabolism 295
Kreb's TCA Cycle 297
Glycogen Metabolism 303
Pentose Phosphate Pathway 308
Mytochondrial Electron Transport System 312
Mitochondrial Transport/Comunication 316

10. **Lipid metabolism** **343**
Oxidation of Fatty Acids 345
Ketone Bodies Metabolism 351
Fatty acid Biosynthesis 357

Triacylglycerol Biosynthesis 364
Regulation of Fatty Acid Metabolism 365

11. Nucleic acid metabolism 379
Purine Metabolism 379
Pyrimidine Metabolism 383
RNA Synthesis 386
DNA Synthesis 397

12. Amino acid and Protein metabolism 427
Protein Catabolism 427
Amino Acid Metabolism 428
Urea Cycle 432
Protein Synthesis 439
Protein Metabolism and Nitrogen Economy 450

13. Photosynthesis 467
The Chloroplast 467
Light Reaction of Photosynthesis 470
Dark Reaction of Photosynthesis 478
Photorespiration 482

14. General Biochemistry Glossry 501

15. Suggested Readings 535

16. Common Important Abbreviations 537

17. Index 545

Chapter 1

Introduction

Biochemistry is the chemistry of living organisms. It bridges the gap between the conventional chemistry and biology. Living organisms have certain extraordinary properties. They can grow, respond to stimuli and replicate themselves with high fidelity. All these activities are ultimately interpretable in chemical terms. The lifeless organic molecules with appropriate complexity and properties make a living thing. The basic phenomena of biochemistry is to understand how the collections of inanimate molecules that constitute living organisms interact with each other to maintain life. The basic life processes or chemistry remains broadly the same whether it is an unicellular microorganism or the higher organisms such as human or plants. Life is nothing but thousands of ordered chemical reactions. In other words, chemistry is the logic of all biological phenomena.

Origin of Life

What is life?. This is not as easy to define as we might like! Life has several properties, none of which are unique or defining, but which together contribute to our understanding of living thing. Life is:

- Improbable (by the 2^{nd} law of thermodynamics).
- Data (DNA is a ternary code).
- Metabolism (complex, autocatalytic biochemistry).
- Replication (self-copying, with heredity).

So when does the first evidence of improbable, information-containing, metabolic replication occur in the fossil record? The Earth is 4,500 million years old, as judged by several corroborating radionuclide studies of the oldest rocks on the planet show. Meteoric bombardment of the proto-Earth continued heavily until 4,000 MYA, probably precluding life during this period. The majority of the oldest rocks on Earth are 3,500 million years old, and the earliest microfossils are from 3,000+ MYA, hence we only have a window of about 500 million years from the end of the meteoric bombardment to the first signs of microbial life. This means we are either very lucky, or life is a high-on certainty!

What did life use as its raw materials? Early theories of life's origins thought that the earth had a reducing atmosphere (*i.e.,* lots of ammonia and methane) but this seems less likely now, as our understanding of early Earth chemistry has proceeded. An oxidizing atmosphere (oxygen) came very much later as evidenced by absence of rust in earliest rocks (the cyanobacteria were responsible for this bit of environmental vandalism), and it's likely that the original atmosphere of Earth was very dull and fairly neutral (nitrogen, carbon dioxide). This is one of the reasons that hydrothermal vents have become popular: life involves highly reduced carbon compounds, and the only places reducing agents are in abiotic abundance are in places like vents where gases escape from the Earth's mantle. In addition to the contributions from the atmosphere (or lack thereof), meteoric waste (amino acids, water-ice, cyanide, polyaromatics) and hydrothermal-associated chemicals (hydrogen sulphide, carbon monoxide, cyanide, pyrite) may have contributed to the alleged 'soup' from which life evolved.

When discussing the first organisms, we *should* distinguish between the most recent common ancestor of life (which may, or may not, have been Archaea-like) and the first forms of life. These may not *necessarily* be the same thing: imagine all life but mammals were wiped out. We might start invoking something shrew-like as the first organism, but this is clearly ridiculous. Similarly, DNA/protein creatures may just be lucky survivors from a far more diverse group of proto-life. However, bearing this in mind, it can be noted that the Archaea (those weird-arse bacteria that live in boiling sulphuric acids, *etc.*) are sulfur metabolisers and hyperthermophiles, supporting a hydrothermal origin, if indeed these features in Archaea are 'primitive', of which we have no guarantee.

The formation of polymers is more problematic. A major difficulty is that biopolymers are all thermodynamically unstable relative to their hydrolysis products. Some theories, but no certainty as to how polymers may have formed, though polymers have been synthesized under conditions which may have occurred on the early Earth.

The biggest problem for the origin of life is the issue of how we go from polymers to living "systems."

- Consider the probelm of protein biosynthesis:
    - Going from DNA (information archive) to RNA (usable information) requires a rather complex system to assure accurate transcription today. However, we could assume a much simpler system in the early, low-competition, Earth.
    - Going from RNA to protein incredibly complex:

        - Need adaptor molecules (t-RNA) because there isn't any natural relationship between RNA codes (sequences) and particular amino acids.
        - Need enzymes to match specific amino acids to specific tRNA's because even the tRNA's are not specific to amino acids in term of recognizing them.
            - as a result, need 20 tRNAs + 20 proteins!
        - Need a complex molecular machine, the Ribosome, to read off the mRNA message using the tRNAs and make proteins:
        - Ribosome consists of
            - 1-2 small RNAs + two large, complexly folded, RNAs,
            - 50-100 accessory proteins.
    - The chance of such a system arising spontaneously is truly infinitesimal.
- "RNA World" has been postulated to solve these dificulties.
    - In this scenario RNA-based life preceeds "modern" life.
    - First "life" combined information and catalytic properties in single RNA-protein molecules.
    - Later there was a transition where proteins took over much of machinery over time.

The Elements of Life

The basic requirements of an idealized living thing is simplest life form and ask why life should use the particular atoms and molecules dominating in a particular living organisms.

Periodic Table of Biologically Important Elements																	
H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
					Mo								Sn			I	

The following observations may be made regarding the elements of life:

> **Life is Largely a Phenomena of Hydrogen and the Second Period of the Periodic Table.** The major component elements are C, H, O, N in all known organisms are from these periods.

- First, It was observed that C, H, O and N are the smallest elements capable of forming 1, 2, 3, and 4 bonds, respectively. Smallest is important because that means they can form the strongest most stable covalent bonds. So these atoms are going to be capable of forming some of the most stable molecules, an important consideration for something that needs to grow and reproduce in a hostile environment.
  - C is particularly noteworthy because it forms strong, stable bonds with itself. As a result it can form the backbone of large chain and branched structures, a unique character among the elements.
- Second, C, N, and O are also the only elements capable of forming strong multiple bonds (carbon and nitrogen can form triple bonds, all three can form double bonds).

The Next Important Elements to Life Occur in Period 3: P and S are the smallest elements capable of multiple covalent bonds to C, O and N, and which also have available *d*-shells. The *d*-shells allow additional transition states and reaction mechanisms. P and S are particularly important in the capture, storage, and distribution of chemical energy.

Conveniently these elements are among the most abundant in the Universe. None-the-less, these elements were *chosen* for their special properties, specifically strong covalent bond formation (to enable the formation of stable biomolecules), the ability of carbon to form large branched molecules, and for C, N, and O the formation of multiple bonds which provides chemical flexibility (step-wise oxidations, different hybridization geometries etc.).

Elemental Ions:

- The "essential" elemental ions found in all studied species, Ca (+2), Mg (+2), K (+1), Na (+1) and Cl (-1) were probably chosen more on the basis of availability in the primordial oceans than for any specific properties: other ions are very similar.
- The trace elements required by all studied organisms, Mn, Fe, Co, Cu, and Zn, are all used as co-catalysts and/or ligands. Thus they were probably chosen for their specific redox properties and/or electronic structures as well as their availability on the early earth.
- A variety of other elements are required by atleast a few organisms, and These are B, F, Al, Ti, V, Cr, Ni, Ga, As, Se, Br, Mo, Sn and I. The elements like He, Li, Be, Ne, Ar, Sc, Ge, Kr are not known to be of biological

importance, but are shown as "place-markers" to help us keep track on the Table.

Biomolecules to Cells

There are a few critically important small molecular precursors to biomolecules found in the environment. Biomolecules can be looked at in two major categories: small molecules and macromolecules.

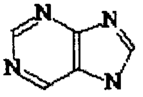
The small molecules are going to be either metabolites or monomers from which the macromolecules are built.

First let's note the "**inorganic**" (sometimes called mineral) molecules and molecular ions: oxygen (O_2), water (H_2O), carbon dioxide (CO_2), ammonia or ammonium ion (NH_3 or NH_4^+), nitrate ion (NO_3^-), nitrogen (N_2), phosphate ion (PO_4^{3-}) and sulphate ion (SO_4^{2-}). These are mostly metabolites, though the ions can also serve as counter ions along with chloride in creating the intracellular media.

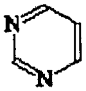
These molecules and ions in turn can be made into metabolites, small organic molecules used in energy transformation and as precursors to monomers and macromolecules.

The **monomers and the associated macromolecules** are divided into four major categories:

1. The nitrogenous bases (purines and pyrimidines) which are components of the nucleic acids (RNA and DNA-used for information storage and processing)

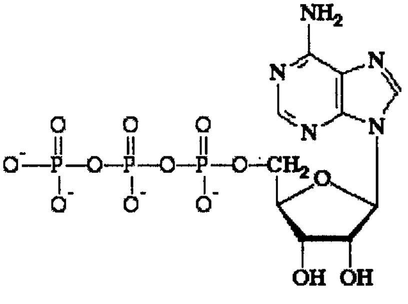


purine ring

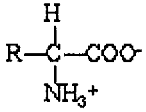


pyrimidine ring

Both purines and pyrimidines are linked to a sugar, ribose or deoxyribose, and phosphate in their active, nucleotide, forms, as in ATP, below:



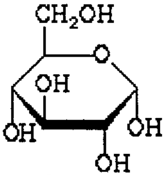
2. The amino acids.



$$R-\overset{\displaystyle H}{\underset{\displaystyle NH_3^+}{C}}-COO^-$$

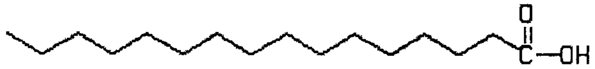
Amino acids are components of proteins, which comprise the machinery of life [enzymes] and much of the structure of life-proteins are the molecules that do things.

3. The sugars, which are components of the polysaccharides (together comprising the carbohydrates, which are used for energy storage and structure). Glucose, the most common sugar, is shown in a cyclic form. Note that a sugar must have an aldehyde or ketone and two or more alcohol functional groups by definition.

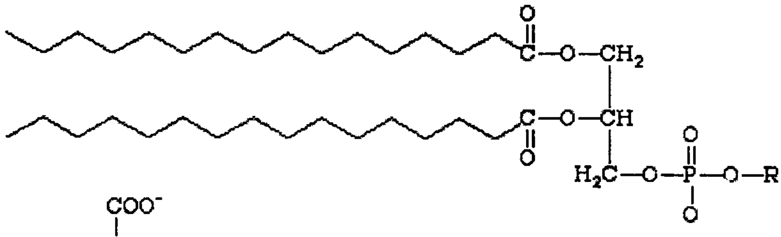


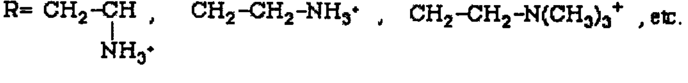
Glucose

4. The fatty acids which, together with glycerol, make up the fats (used mostly for energy storage) and the phospholipids (the major component of cell membranes). The 16 carbon fatty acid palmitate is shown below:



A typical phospholipid is shown here, replacement of the phosphate ester group with a third fatty acid would give a fat instead:





$$R = CH_2-\overset{\displaystyle COO^-}{\underset{\displaystyle NH_3^+}{CH}}\ ,\quad CH_2-CH_2-NH_3^+\ ,\quad CH_2-CH_2-N(CH_3)_3^+\ ,\text{etc.}$$

The amino acids, nucleotides, and sugars can all be polymerized to give the macromolecules characteristic of life: proteins, nucleic acids, and polysaccharides, respectively. Briefly, proteins comprise the machinery and much of the structure of life; nucleic acids provide the information required to specify the proteins, and polysaccharides provide structural fibers and energy storage molecules.

Note that all of these families of molecules exhibit chirality's in some, and generally most, of their members. Also, biological systems chose a single chirality for each family (e.g., L-amino acids, D-sugars)

All of these molecules together go to make up cells.

Cells and Organelles

There are two main cell types: prokaryote and eukaryote.

Prokaryote Cell

The structure of a prokaryote is very much simpler than that of a eukaryote. There are no endomembranes, endosymbionts, nucleus or cytoskeleton. The DNA is carried on the genophore, a circular chromosome, in a ill defined area of the cytosol called the nucleoid. The chromosome is attached to the cell membrane during cell division (fission), frequently at a point called the mesosome.

- *pro-karyon* - before kernel.
- No nucleus - free circular genophore.
- 'Simple' rotating motor-type flagellum.
- Cell division by fission, genophore attached to plasmalemma by mesosome.
- Few membrane-bound organelles, no double-membrane bound organelles.
- They are 'small' because diffusion limits the rate of transport across the cell - 1 μm.

Bacteria are prokaryotes, lacking well-defined nuclei and membrane-bound organelles, (Fig. 1.1) and with chromosomes composed of a single closed DNA circle. They come in many shapes and sizes, from minute spheres, cylinders and spiral threads, to flagellated rods, and filamentous chains. They are found practically everywhere on Earth and live in some of the most unusual and seemingly inhospitable places.

Evidence shows that bacteria were in existence as long as 3.5 billion years ago, making them one of the oldest living organisms on the Earth. Even older than the bacteria are the archeans (also called archaebacteria) tiny prokaryotic organisms that live only in extreme environments: boiling water, super-salty pools, sulphur-spewing volcanic vents, acidic water, and deep in the Antarctic ice. Many scientists now believe that the archaea and bacteria developed separately from a common ancestor nearly four billion years ago. Millions of years later, the ancestors of today's eukaryotes split off from the archaea. Despite the superficial resemblance to bacteria, biochemically and genetically, the archea are as different from bacteria as bacteria are from humans.

In the late 1600s, Antoni van Leeuwenhoek became the first to study bacteria under the microscope. During the nineteenth century, the French scientist Louis Pasteur and the German physician Robert Koch demonstrated the role of bacteria as pathogens (causing disease). The twentieth century saw numerous advances in bacteriology, indicating their diversity, ancient lineage, and general importance. Most notably, a number of scientists around the world made contributions to the field of microbial ecology, showing that bacteria were essential to food webs and for the overall health of the Earth's ecosystems. The discovery that some bacteria produced compounds lethal to other bacteria led to the development of antibiotics, which revolutionized the field of medicine.

There are two different ways of grouping bacteria. They can be divided into three types based on their response to gaseous oxygen. Aerobic bacteria require oxygen for their health and existence and will die without it. Anerobic bacteria can't tolerate gaseous oxygen at all and die when exposed to it. Facultative aneraobes prefer oxygen, but can live without it.

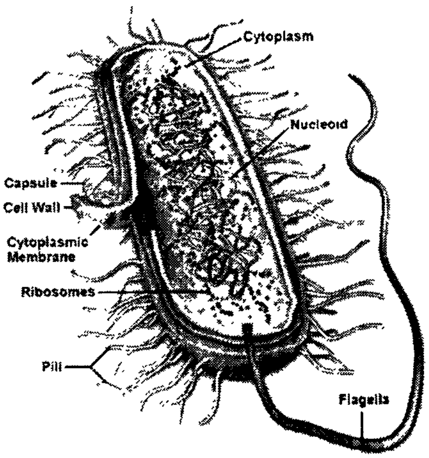


Fig. 1.1 : Prokaryotic Cell Structure

The second way of grouping them is by how they obtain their energy. Bacteria that have to consume and break down complex organic compounds are heterotrophs. This includes species that are found in decaying material as well as those that utilize fermentation or respiration. Bacteria that create their own energy, fueled by light or through chemical reactions, are autotrophs.

Capsule - Some species of bacteria have a third protective covering, a capsule made up of polysaccharides (complex carbohydrates). Capsules play a number of roles, but the most important are to keep the bacterium from drying out and to protect it from phagocytosis (engulfing) by larger microorganisms. The capsule is a major virulence factor in the major disease-causing bacteria, such as Escherichia coli and Streptococcus pneumoniae. Nonencapsulated mutants of these organisms are avirulent, i.e., they don't cause disease.

Cell Envelope - The cell envelope is made up of two to three layers: the interior cytoplasmic membrane, the cell wall, and -- in some species of bacteria -- an outer capsule.

Cell Wall - Each bacterium is enclosed by a rigid cell wall composed of peptidoglycan, a protein-sugar (polysaccharide) molecule. The wall gives the cell its shape and surrounds the cytoplasmic membrane, protecting it from the environment. It also helps to anchor appendages like the pili and flagella, which originate in the cytoplasm membrane and protrude through the wall to the outside. The strength of the wall is responsible for keeping the cell from bursting when there are large differences in osmotic pressure between the cytoplasm and the environment.

Cell wall composition varies widely amongst bacteria and is one of the most important factors in bacterial species analysis and differentiation. For example, a relatively thick, meshlike structure that makes it possible to distinguish two basic types of bacteria. A technique devised by Danish physician Hans Christian Gram in 1884, uses a staining and washing technique to differentiate between the two forms. When exposed to a gram stain, gram-positive bacteria retain the purple colour of the stain because the structure of their cell walls traps the dye. In gram-negative bacteria, the cell wall is thin and releases the dye readily when washed with an alcohol or acetone solution.

Cytoplasm - The cytoplasm, or protoplasm, of bacterial cells is where the functions for cell growth, metabolism, and replication are carried out. It is a gel-like matrix composed of water, enzymes, nutrients, wastes, and gases and contains cell structures such as ribosomes, a chromosome, and plasmids. The cell envelope encases the cytoplasm and all its components. Unlike the eukaryotic (true) cells, bacteria do not have a membrane enclosed nucleus. The chromosome, a single, continuous strand of DNA, is localized, but not contained, in a region of the cell called the nucleoid. All the other cellular components are scattered throughout the cytoplasm.

Cytoplasmic Membrane - A layer of phospholipids and proteins, called the cytoplasmic membrane, encloses the interior of the bacterium, regulating the flow of materials in and out of the cell. This is a structural trait bacteria share with all other living cells; a barrier that allows them to selectively interact with their environment. Membranes are highly organized and asymmetric having two sides, each side with a different surface and different functions. Membranes are also dynamic, constantly adapting to different conditions.

One of those components, plasmids, are small, extrachromosomal genetic structures carried by many strains of bacteria. Like the chromosome, plasmids are made of a circular piece of DNA. Unlike the chromosome, they are not involved in reproduction. Only the chromosome has the genetic instructions for initiating and carrying out cell division, or binary fission, the primary means of reproduction in bacteria. Plasmids replicate independently of the chromosome and, while not essential for survival, appear to give bacteria a selective advantage.

Plasmids are passed on to other bacteria through two means. For most plasmid types, copies in the cytoplasm are passed on to daughter cells during binary fission. Other types of plasmids, however, form a tubelike structure at the surface called a pilus that passes copies of the plasmid to other bacteria during conjugation, a process by which bacteria exchange genetic information. Plasmids have been shown to be instrumental in the transmission of special properties, such as antibiotic drug resistance, resistance to heavy metals, and virulence factors necessary for infection of animal or plant hosts. The ability to insert specific genes into plasmids have made them extremely useful tools in the fields of molecular biology and genetics, specifically in the area of genetic engineering.

Flagella - Flagella (singular, flagellum) are hairlike structures that provide a means of locomotion for those bacteria that have them. They can be found at either or both ends of a bacterium or all over its surface. The flagella beat in a propeller-like motion to help the bacterium move toward nutrients; away from toxic chemicals; or, in the case of the photosynthetic cyanobacteria; toward the light.

Nucleoid - The nucleoid is a region of cytoplasm where the chromosomal DNA is located. It is not a membrane bound nucleus, but simply an area of the cytoplasm where the strands of DNA are found. Most bacteria have a single, circular chromosome that is responsible for replication, although a few species do have two or more. Smaller circular auxiliary DNA strands, called plasmids, are also found in the cytoplasm.

Pili - Many species of bacteria have pili (singular, pilus), small hairlike projections emerging from the outside cell surface. These outgrowths assist the bacteria in attaching to other cells and surfaces, such as teeth, intestines, and rocks. Without pili, many disease-causing bacteria loose their ability to infect because they're unable to attach to host tissue. Specialized pili are used for conjugation, during which two bacteria exchange fragments of plasmid DNA.

Ribosomes - Ribosomes are microscopic "factories" found in all cells, including bacteria. They translate the genetic code from the molecular language of nucleic acid to that of amino acids—the building blocks of proteins. Proteins are the molecules that perform all the functions of cells and living organisms. Bacterial ribosomes are similar to those of eukaryotes, but are smaller and have a slightly different composition and molecular structure. Bacterial ribosomes are never bound to other organelles as they sometimes are (bound to the endoplasmic reticulum) in eukaryotes, but are free-standing structures distributed throughout the cytoplasm. There are sufficient differences between bacterial ribosomes and eukaryotic ribosomes that some antibiotics will inhibit the functioning of bacterial ribosomes, but not a eukaryote's, thus killing bacteria but not the eukaryotic organisms they are infecting.

- Large 50S subunit:
    - 23S rRNA,
    - 5S rRNA,
    - 34 proteins.
- Small 30S subunit:
    - 16S rRNA,
    - 21 proteins.

Biochemical composition of **prokaryote bacterial cell** : 70% water, 15% protein, 7% nucleic acids, 3% polysaccharides, 3%, lipids, 1% inorganic ions, & 0.2% metabolites.

Eukaryote Cell

The most characteristic feature of a eukaryotic cell, the nucleus, consists of a nucleoplasm surrounded by a double nuclear membrane pierced by nuclear pores. The nucleoplasm contains the (linear) chromosomes of the cell, which are organised into heterochromatin, which stains only a little, and euchromatin, which stains more densely. The most important euchromatic area is the nucleolus, in which ribosomes are formed (Fig. 1.2).

- *eu-karyon* - true kernel.
- Double-membrane bound nucleus containing linear chromosomes.
- Complex 9+2-type undulipodium, cytoskeleton, cytosis and mitosis.
- Many membrane-bound organelles and double membrane-bound endosymbionts.

- They can grow 'large' because cytoplasmic streaming allows rapid transport across the cell - 100 μm.

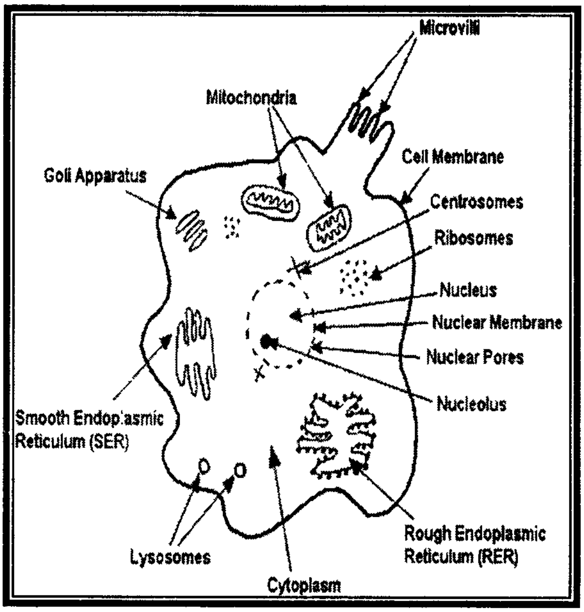

Microvilli
Mitochondria
Goli Apparatus
Cell Membrane
Centrosomes
Ribosomes
Nucleus
Nuclear Membrane
Nuclear Pores
Nucleolus
Smooth Endoplasmic Reticulum (SER)
Rough Endoplasmic Reticulum (RER)
Lysosomes
Cytoplasm

Fig. 1.2 : Typical Animal Cell - Eukaryotes

The role of the nucleus is three-fold:

- Storage and protection of the genome.
- Regulation of gene expression.
- Creation of ribosomes.

DNA storage takes up about 10% of the cell of both prokaryotes (nucleoid area) and eukaryotes (nucleus). However, don't forget that some of the genome lives elsewhere: plasmids, endosymbionts (mitochondria), *etc.* The nucleus of eukaryotes also protects the genome from the cytoskeleton. Condensation of genome during mitosis is required to withstand these stresses. This is not relevant in prokaryotes, as they lack a cytoskeleton. The existence of the nucleus permits processing of mRNA, and therefore defers translation. Alternative splicing can be performed to generate different proteins from the same RNA primary transcript. mRNA is capped and tailed to permit it to exit the nucleus. rRNA and tRNA are also heavily processed by RNA editing, *i.e.,* base modification (this also occurs to some mRNA in trypanosomes). rRNA modification occurs in the nucleolus, where ribosomes are constructed.

The nucleus is bound by a double-membrane, which is contiguous through the nuclear pores, known as the nuclear envelope. The pores are required to allow RNA out and membrane lipids in (which is needed for growth during S phase).

The inner face of the inner nuclear envelope (INE) is coated by the nuclear lamina, which contains intermediate fibres called lamins A, B and C (atleast in mammals). Phosphorylation of lamins by kinases cause nuclear envelope breakdown during prometaphase. Chromosomes occupy definite positions within the nucleus because of the interaction between lamins and telomeres, for example the Rabl conformation in yeast.

The outer nuclear envelope (ONE) is surrounded by other intermediate fibres, and is essentially just the RER surrounding the nucleus, and continuous with it. The space between the INE and ONE is termed the perinuclear space, and is continuous with the RER cisternae.

The cell wall of eukaryotes (when present) is usually composed of a β-(1, 4)-glucan of some sort. In fungi, it is mostly chitin (*N*-acetylaminoglucan), in plants cellulose, but more exotic ingredients are common. Animal cells lack a wall, but may have a glycocalyx, which is a layer of thickened glycoproteins surrounding them and connecting them to the extracellular matrix.

Almost all eukaryotes have mitochondria, which are the remains of bacteria that became endosymbionts of the eukaryotic cell about a billion years ago. They perform oxidative phosphorylation and generate energy in the form of ATP for the cell. They have their own 70S ribosomes and some of their own DNA. Mitochondria have a double membrane surrounding them, the inner one is highly folded into cristae, surrounding a matrix space.

The fraction of eukaryotic ribosomes are as under -

- Large 60S subunit:
    - 28S rRNA,
    - 5S rRNA,
    - 5.8S rRNA,
    - 49 proteins.
- Small 30S subunit:
    - 18S rRNA,
    - 33 proteins.

Eukaryotic DNA is bound by histones, and requires some degree of unpacking for expression. Much of their genome is composed of parasitic DNA and introns. Three RNA polymerases exists, (approximately) one for each sort of major RNA product:

- RNApol-I - rRNA.
- RNApol-II - mRNA and snRNA.
- RNApol-III - tRNA and 5S rRNA.

RNA is heavily processed in the nucleus, which allows deferred translation. They possess large 80S ribosomes. Organelles of Eukaryote and it's function are summarized in Table 1.1. However, relative volume of organelles of liver cells are given in Table 1.2.

Table 1.1 : Organelle of Eukaryote and its Function in the Cell

Organelle	Structure/Function
Cell Membrane	The cell membrane keeps the cell together by containing the organelles within it. Cell membranes are selectively-permeable, allowing materials to move both into and outside of the cell. Active and passive transport systems; receptors and signal processing systems (synthesis of various second messengers etc.).
Centrosomes	The centrosomes contain the centrioles, which are responsible for cell-division.
Cytoplasm	Cytoplasm is a jelly-like substance that is sometimes described as "the cell-matrix". It holds the organelles in place within the cell. Site for Glycolysis and most of gluconeogenesis; Pentose Phosphate shunt; Fatty acid biosynthesis.
Goli Apparatus	The goli apparatus of a cell is usually connected to an endoplasmic reticulum (ER) because it stores and then transports the proteins produced in the ER. Further modification of membrane and export proteins.
Glycogen Granules	Enzymes of glycogen synthesis and breakdown including branching and debranching.
Lysosomes	Lysosomes are tiny sacs filled with enzymes that enable the cell to utilize its nutrients. Lysosomes also destroy the cell after it has died, though there are some circumstances (diseases/conditions) in which lysosomes begin to 'break-down' living cells. Hydrolytic (digestive) enzyme localization.
Microvilli	"Microvilli" is the pural form; "Microvillus" is the singular form. Microvilli are finger-like projections on the outer-surface of the cell. Not all cells have microvilli. Their function is to increase the surface area of the cell, which is the area through which diffusion of materials both into, and out of, the cell is possible.
Mitochondria	"Mitochondria" is a plural term; which is appropriate as these are not found alone. The quantity of mitochondria within cells varies with the type of cell.

	These are the energy producers within the cell. They generate energy in the form of Adenosine Tri-Phosphate (ATP). Generally, the more energy a cell needs, the more mitochondria it contains. Site for Kreb's Citric Acid Cycle; Electron transport system and Oxidative Phosphorylation; Fatty acid oxidation; Amino acid catabolism; Interconversion of carbon skeletons.
Nuclear Membrane	The nuclear membrane separates the nucleus and the nucleolus from the rest of the contents of the cell.
Nuclear Pore	Nuclear pores permit substances (such as nutrients, waste, and cellular information) to pass both into, and out of, the nucleus.
Nucleolus	The nucleolus is responsible for the cell organelles (e.g., lysosomes, ribosomes, etc.). Localized region of the nucleus in which ribosomal RNA's are synthesized and processed.
Nucleus	The nucleus is the "Control Center" of the cell, which contains DNA (genetic information) in the form of genes, and also information for the formation of proteins. Information is carried on chromosomes, which are a form of DNA. Site for DNA replication, synthesis and processing of messenger RNA's.
Peroxisomes	Amino acid oxidases, catalase-oxidative degradation reactions.
Ribosomes	Ribosomes interpret cellular information from the nucleus and so synthesize appropriate proteins, as required.
Rough Endoplasmic Reticulum (RER)	"Rough" indicates that there are ribosomes attached to the surfaces of the endoplasmic reticulum. The endoplasmic reticulum is the site for membrane and secretary protein biosynthesis.
Smooth Endoplasmic Reticulum (SER)	"Smooth" indicates that there are no ribosomes attached to the surfaces of the endoplasmic reticulum. Site of phospholipid biosynthesis and detoxification reactions takes place.

Table 1.2. : Relative Volumes of Compartments in a Liver Cell:

Organelle	% Total Volume	Approx. Number per Cell
Cytosol	54	1
Mitochondria	22	1700
Rough Endoplasmic Reticulum	9	1
Smooth Endoplasmic Reticulum + Golgi	6	
Nucleus	6	1
Peroxisomes	1	400
Lysosomes	1	300
Endosomes	1	200

Data from: Alberts, *et al.* Molecular Biology of the Cell, 1994.

Plant Cell

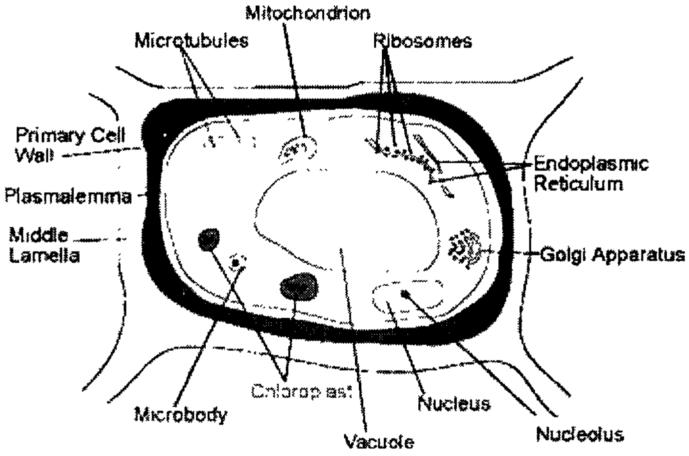

Mitochondrion
Microtubules
Ribosomes
Primary Cell Wall
Plasmalemma
Middle Lamella
Endoplasmic Reticulum
Golgi Apparatus
Microbody
Chloroplast
Nucleus
Vacuole
Nucleolus

Fig. 1.3 : Plant Cells Structure

All of the organelles described in animal cell are here as well, but with a few additions: (Fig. 1.3; Table 1.3.)

Table 1.3 : Specialized organelles of Plant Cell and Its Functions

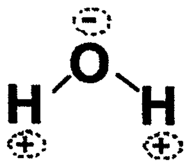
Organelle	Structure/Function
Chloroplasts	Light capturing processes and electron transport & oxidative phosphorylation for photosynthesis; Calvin cycle (dark reactions of photosynthesis).
Glyoxisomes	Location of glyoxalate cycle.
Cell wall	Made up of cellulose glued together with lignin (a plastic like polymer) - maintains cell integrity against high osmotic pressure, gives cell rigidity.
Vacuole	Storage of dilute aqueous solutions, provides fluid for osmotic pressure.

Water

Water is a very unusual, even incredible substance whose amazing properties are often unappreciated because of its ubiquitousness. Water's special properties include extremely high MP and BP (0 °C & 100 °C K, compare to methane, -183 °C & -161 °C, with a MW of 16 vs. water's 18); a high heat capacity (18 cal/°C mol vs. 8 cal/°C mol for methane); it has a high viscosity; its solid form is less dense than the liquid form at the same temperature (ice floats on water - very rare), it has a large surface tension, and it has a high dielectric constant (78.5 vs. 1.9 for hexane).

The high MP, BP, and heat capacity of water all predict relatively strong bonding between water molecules, so let's first review the types of bonding which occur between atoms and molecules. The most stable bonds are of course covalent bonds (with bond energies of 50 [S-S] to 80 [C-C] to 110 [O-H] kcal/mol), occurring when It has significantly overlap of atomic orbitals.

Water of course is a covalent structure: H-O-H. The special properties of water is -polarity of between O-H bonds and the resultant dipole moments of the bonds and the molecule itself.



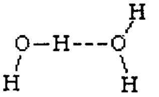
The water molecule itself is bent, with an angle of 104.5° between the hydrogens (compare to 109.5° for sp^3 tetrahedron).

Because of the very strong dipole moments of these bonds and the very small size of the hydrogen substituents on water, a slight degree of orbital overlap occurs between adjacent water oxygens and hydrogens to give partial covalent bonds known as H-bonds (effectively, can only form with O, N, & F).

- Note that the partial covalent character means that they are directional! Compare the bond length of water H bonds (0.18 nm) to the covalent bond-length between O and H of 0.096 nm - notice that the bond distance is nearly twice the true covalent bond distance, but significantly less than the van der Waals radius of 0.26 nm.

In addition to covalent bonds and H-bonds there are a variety of non-covalent bonds/interactions as seen in the Table 1.4:

Table 1.4 : Non-covalent Bond/Interations with Examples

Interaction Type	Example	Average Strength, kcal/mol (kJ/mol)	Range**
Charge-charge (ionic)	—NH_3^+ Cl^-	5 (20) [in water solution]	1/r
Charge-dipole	—NH_3^+ $ClCH_3$	-	1/r^2
Dipole-dipole	$ClCH_3$ $ClCH_3$	-	1/r^3
Charge-induced dipole	Na^+ CH_4	-	
Dipole-induced dipole*	CH_4 $ClCH_3$	0.1-0.2 (0.4-4)	1/r^6
Dispersion*	CH_4 CH_4	0.1-0.2 (0.4-4)	1/r^6
Hydrogen bond		3-8 (12-30)	
van der Waals repulsion	-	-	1/r^{12}

Source : *van der Waals interactions, **from Zubay *Biochemistry* 3rd. pp. 89.

Within solid bulk water (ice):

- Every water molecule is bonded to 4 others, as in the ice structure
    - In liquid water the molecules are still bonded to a large degree (the heat of fusion for ice is only 13% of the heat of vapourization for ice, thus most of the H-bonds must survive melting).
    - Of course in liquid water the bonds are very unstable (average lifetime about 10 psec = 10^{-11} sec), exchanging constantly to give a "flickering cluster" structure.
    - The various properties of water arise from this structure. (Note high BP & MP, heat cap., viscosity, and, less obviously, that ice floats.
- Ice floats because the molecules are in an open lattice rather than close-packed. Garrett and Grisham (in their text, *Biochemistry,* 2nd ed.) note that close-packed water molecules would only occupy about 57% of the volume of ice. This would lead one to expect that ice would float "high." It doesn't because most of the structure remains in the liquid phase at 0° C.)

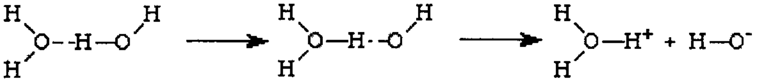
Water is an Excellent Solvent for Polar Substances since its dipolar structure enables it to insulate them from each other and it can make good dipole-dipole and dipole-charge bonds. Anything which can H-bond will also of course be quite soluble.

How does Water Interact with Non-Polar Molecules?

- The problem here is that in order to dissolve in water a non-polar molecule must disrupt a series of H-bonds and no new bonds of equal strength are substituted.
- Thus water tends to **exclude** non-polar substances. When we forcefully disperse a non-polar substance into water then the water must form a cage around the molecule to maximize H-bonds for each water molecule.
    - Now an additional problem arises-the waters hydrating the non-polar group are "locked" in place - they can't easily flip about because there is no interior bond for substitution!
- Thus the **entropy** of these water molecules is greatly reduced. So **the insolubility of non-polar groups in water has both enthalpic and entropic factors!**

Ionization of Water, pH & Buffers

Dissociation of Water Molecules: In normal aqueous solution, there is a certain probability that a hydrogen nucleus (a proton) can exchange between two hydrogen bonded molecules:



(Of course the hydronium ion, H_3O^+, will be associated with additional water molecules as well through H-bonding. For simplicity we will just write H^+, with the understanding that it refers infact to hydrated hydronium ions in aqueous solution.) Note the reaction is not highly favoured, in neutral solution (no excess H^+or OH^-) there will only be 10^{-7} molar hydronium ions, in other words only about 2 of every billion water molecules will be protonated!

For aqueous solution $[H^+][OH^-] = 10^{-14}$;

- pH = -log $[H^+]$. Remember that a low pH means a high concentration of protons.
- $pK_a = -\log K_a$therefore pH + pOH = 14, where pOH = -log$[OH^-]$.
  - The Brönsted definition for acids and bases: An acid is a proton donor, while a base is a proton acceptor. Recall the corollary that acids and bases therefore exist as conjugate acid base pairs. Note that when an acid by this definition gives up a proton it becomes a base, since the reverse reaction would be accepting a proton:

$$\underset{\text{acid}}{CH_3COOH} + \underset{\text{base}}{OH^-} \longrightarrow \underset{\text{conjugate base}}{CH_3COO^-} + \underset{\text{conjugate acid}}{HOH}$$

$$\underset{\text{base}}{CH_3COO^-} + \underset{\text{acid}}{HOH} \longrightarrow \underset{\text{conjugate acid}}{CH_3COOH} + \underset{\text{conjugate base}}{OH^-}$$

Thus the acetic acid in the first reaction becomes its **conjugate base** acetate ion, while the base, hydroxide ion, becomes its **conjugate acid,** water. In the reverse reaction the nomenclature also reverses. Note that a molecule such as water can be both an acid, donating a proton to become its conjugate base hydroxide ion, or it can be a base, accepting a proton to become its conjugate acid, a hydronium ion.

pH & Buffers

The strengths (ability to donate protons) of acids vary considerably.

- For the general acid HA we can write:

$$HA + H_2O \longleftrightarrow A^- + H_3O^+$$

$$K_a = \frac{[H_3O^+][A^-]}{[HA]}$$

Where K_a is the acid dissociation constant. (Note that the definition of K_a is based on the Brønsted definition.) Values of K_a can vary tremendously (10^{15} to 10^{-60}) - after all anything with at least one proton can be considered an acid under some circumstances with this definition. The common definition of a strong acid is an acid which dissociates completely in a 1 M solution. The common strong acids in aqueous solution, such as sulphuric, nitric and hydrochloric acids have K_a values (for the first dissociation in the case of sulfuric) of 10^2 to 10^9. Thus they all dissociate completely (first dissociation only for sulphuric) in aqueous solution, though they will have different strengths in some other solvents. Most common organic acids are weak in aqueous solution, having K_a values of 10^{-5} to 10^{-15}. Note that whether an acid is strong or weak is dependent on the solvent system! Strong acids have weak conjugate bases, and vice-versa.

- For reactions involving a strong acid or base we can assume, for practical purposes, that all of the strong acid or base added to a mixture will react until the base or acid originally present in solution is completely consumed. (Of course this is an approximation, all reactions actually approach an equilibrium condition, so that, in theory, there is always some reactant and some product present.) For example, if we start with a solution containing 0.100 mole of acetic acid and add 0.050 moles of sodium hydroxide the resulting mixture will contain 0.050 moles acetic acid, 0.050 moles sodium acetate and 0.000 moles sodium hydroxide (actually about 10^{-10} moles, which is 0.000 for our thousandths place significant figure calculation).

The equilibrium equation for a mixture of a weak acid and its conjugate base can be rewritten by taking logs of both sides and rearranging to give the Henderson-Hasselbalch equation: $pH = pK_a + \log [A^-]/[HA]$

History of Biochemistry

Only during 17th and 18th centuries, important foundations were laid in many fields of biology. The 19th century observed the development of very crucial concepts, which include the cell theory by Schleiden and Schwann, Mendel's study of inheritance and Darwin's theory of evolution. The real push to biochemistry was given in 1828 when total synthesis of urea from lead cyanate and ammonia was successfully achieved by Wohler who thus initiated the synthesis of organic compound from inorganic compound. Louis Pasteur, during 1857, did a great deal of work on fermentations and pointed out categorically the central importance of enzymes in this process. The break through in enzyme research and hence, biochemistry was made in 1897 by Edward Buckner when he extracted enzyme from yeast cells in crude form which could ferment a sugar molecule into alcohol. Neuberg introduced the term biochemistry in 1903.

The early part of 20th century witnessed a sudden outburst of knowledge in chemical analysis, separation methods, electronic instrumentation for biological studies (X-ray diffraction, electron microscope, etc.) which ultimately resulted in understanding the structure and function of several key molecules involved in life processes such as proteins, enzymes, DNA and RNA.

In 1926, James Sumner established the protein nature of enzyme. He was responsible for the isolation and crystallization of urease, which provided a breakthrough in studies of the properties of specific enzymes.

The first metabolic pathway elucidated was the glycolytic pathway during the first half of the 20th century by Embden and Meyerhof. Otto Warburg, Cori and Parnas also made very important contributions relating to glycolytic pathway. Krebs established the citric acid and urea cycles during 1930-40. In 1940, Lipmann described the central role of ATP in biological systems.

The biochemistry of nucleic acids entered into a phase of exponential growth after the establishment of the structure of DNA in 1953 by Watson and Crick followed by the discovery of DNA polymerase by Kornberg in 1956. From 1960 onwards, biochemistry plunged into an interdisciplinary phase sharing much in common with biology and molecular genetics.

Frederick Sanger's contributions in the sequencing of protein in 1953 and nucleic acid in 1977 were responsible for further developments in the field of protein and nucleic acid research.

The growth of biochemistry and molecular biology was phenomenal during the past two decades. The development of recombinant DNA research by Snell and coworkers during 1980 allowed for further growth and emergence of a new field, the genetic engineering.

Thus, there was progressive evolution of biology to biochemistry and then to molecular biology, genetic engineering and biotechnology. The chronological development of biochemistry and other related fields are given in Table 1.5.

Table 1.5 : Important Scientists and their Contribution to Biochemistry and other Related Fields.

1780-1789	Lavoisier	Recognized that respiration is oxidation and first measured oxygen consumption by human subject.
1828	Wohler	Synthesized the first organic compound, urea from inorganic components.
1837	Berzelius	Postulated the catalytic nature of fermentation. He also identified lactic acid as a product of muscle activity.
1838	Schleiden and Schwann	Enunciated the cell theory.
1854-1864	Louis Pasteur	Proved that fermentation is caused by microorganisms.
1866	Mendel	Reported the principles of segregation and independent assortment of genes.
1869	Miescher	Discovered DNA.
1877	Kuhne	Proposed the term 'Enzyme'.
1894	Emil Fischer	Demonstrated the specificity of enzymes and the lock and key relationship between enzyme and substrate.
1897	Buckner	Discovered alcoholic fermentation in cell-free yeast extract.
1902	Emil Fischer	Demonstrated that proteins are polypeptides.
1903	Neuberg	First used the term 'biochemistry'.
1905	Harden and Young	Showed the requirement of phosphate in alcoholic fermentation and identified first coenzyme, cozymase, later shown to be NAD.
1912	Neuberg	Proposed chemical pathway for fermentation.
1913	Michaelis and Menten	Developed kinetic theory of enzyme action.
1926	Sumner	First crystallized an enzyme, urease and proved it to be a protein.
1933	Embden Meyerhof and Parnas	Demonstrated crucial intermediates in the chemical pathway of glycolysis and fermentation.
1937	Krebs	Discovered citric acid cycle.
1940	Lipmann	Role of ATP in biological systems.
1940	Beadle and Tatum	Deduced one gene-one enzyme relationship.

1944	Avery, MacLeod and McCarty	Demonstrated that bacterial transformation was caused by DNA.
1948	Calvin and Benson	Discovered that phosphoglyceric acid is an early intermediate in photosynthetic CO_2 fixation.
1950	Pauling and Corey	Proposed the α-helix structure for keratins.
1950-1953	Chargaff	Discovered the base composition of DNA.
1953	Sanger and Thompson	Determined the complete amino acid sequence of insulin.
1953	Watson and Crick	Proposed the double-helical model for DNA structure.
1954	Arnon and Colleagues	Discovered photosynthetic phosphorylation.
1956	Kornberg	Discovered DNA polymerase.
1958	Meselson and Stahl	Confirmed the Watson-Crick model of semi conservative replication of DNA.
1960	Hamilton and Daniel Nathans	Restriction endonucleases.
1961	Jacob & Monod	Proposed the operon hypothesis and postulated the function of messenger RNA.
1961	Nirenberg and Matthaei	Reported that polyuridylic acid codes for phenylalanine and this opened the way to identification of genetic code.
1961-1965	Nirenberg Khorana and Ochoa	Identified the genetic code words for amino acids.
1969	Arber	Restriction endonucleases.
1977	Sanger	Determination of DNA sequence.
1980	Snell	Development of recombinant DNA research leading to genetic engineering.
1984	Kary Mullis	Polymerase chain reaction.
1997	Wilmut	Viable offspring derived from fetal and adult mammalian cells.
1999	Ingo potrykus	Golden rice rich in β-carotene.

Thus, biochemistry focuses on a limited range of areas within the manifestation of life, which cover -

1. The chemical properties and 3-D structures of biomolecules.
2. The interactions of biomolecules with each other and with inorganic molecules and ions.

3. The synthesis and degradation of substances by organisms.
4. Energy use and storage by organisms.
5. The organization and regulation of biochemical systems.
6. The molecular mechanisms of the storage, transmission and expression of biological information.

QUIZ

The Chemical Basis of Life

Complete each sentence or statement.

1. A/An ________ is an element that is present in small quantities in a living organism.

   Answer: trace element

2. ________ is the three-dimensional shape of a molecule that is attained through rotation of its bonds.

   Answer: Conformation

3. A macromolecule that consists of one or more polypeptide chains is a/an ________.

   Answer: protein

4. All bacteria are ________, unicellular organisms that lack a membrane-bounded nucleus.

   Answer: prokaryotes

5. A/An ________ is a structural unit from which a polymer is built.

   Answer: monomer

6. A/An ________ is any member of a broad class of macromolecules that are largely or wholly hydrophobic.

   Answer: lipid

7. A thermodynamic process that has a net increase in free energy is called a/an ________ and can occur only with the input of free energy from outside the system.

   Answer:nonspontaneous process or endergonic process

8. In a/an ________ reaction, a substance loses electrons.

   Answer:oxidation

9. ________ is the evolutionary process by which the continued existence of a replicating entity depends on its ability to survive and reproduce under the existing conditions.

   Answer: Natural selection

10. A monosaccharide is a/an ________ consisting of a single sugar molecule.

    Answer: carbohydrate

11. The major ________ are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

Answer: nucleic acids

12. During DNA ________, the parental polynucleotide strands separate so that each can direct the synthesis of a complementary daughter strand resulting in two complete DNA double helices.

Answer: replication

13. ________ is a measure of the degree of randomness or disorder of a system.

Answer: Entropy

14. A polypeptide is a polymer consisting of ________ that are linked in linear fashion by peptide bonds.

Answer:amino acid residues

15. A/An ________ organism requires oxygen.

Answer: aerobic

16. A molecule that pairs in a reciprocal fashion with another is called a/an ________.

Answer: complement

17. ________ is a thermodynamic quantity that is taken to be equivalent to the heat content of a biochemical system.

Answer: Enthalpy

18. Bacteria and ________ are the two major types of prokaryotes.

Answer: archaea

19. An example of a nonspontaneous process is a/an ________ reaction that has an overall positive free energy change.

Answer: endergonic

20. In a/an ________ reaction, a substance gains electrons.

Answer: reduction

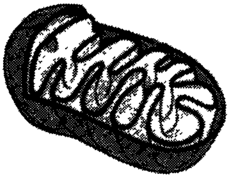
Cells and Organells

Identify the letter of the choice that best completes the statement or answers the question.

1. What part of the cell is responsible for breaking down and digesting things?
   A. Ribosomes
   B. Lysosomes
   C. Endoplasmic Reticulum
   D. Vacuole

Answer: B

2. Identify the organelle pictured.
   - A. Chloroplast
   - B. Endoplasmic Reticulum
   - C. Golgi Apparatus
   - D. Mitochondria



Answer: D

3. What part of the cell serves as the intracellular highway?
   - A. Endoplasmic Reticulum
   - B. Golgi Apparatus
   - C. Cell Membrane
   - D. Mitochondria

Answer: A

4. Which of the following would you NOT find in a bacterial cell?
   - A. DNA
   - B. Cell Membrane
   - C. Golgi apparatus
   - D. Ribosomes

Answer: C

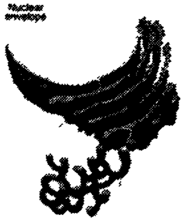
5. Which of the following is found in plant cells, but not animal cells?
   - A. Cell Wall
   - B. Vacuole
   - C. Mitochondria
   - D. Endoplasmic reticulum

Answer: A

6. The jelly like interior of the cell is called the:
   - A. Vacuole
   - B. Cytoplasm
   - C. Cytoskeleton
   - D. Nucleus

Answer: B

7. Identify the organelle.
   - A. Golgi Apparatus
   - B. Endoplasmic Reticulum
   - C. Mitochondria
   - D. Lysosome



Answer: B

8. What part of the cell makes proteins?
   - A. Ribosomes
   - B. Mitochondria
   - C. Lysosomes
   - D. Vacuole

Answer: A

9. Where are ribosomes usually located in animal and plant cells?
   - A. Inside the nucleus
   - B. Near the cell membrane
   - C. On the endoplasmic reticulum
   - D. Inside the vacuole

Answer: C

10. What part of the cell serves to process, package and export proteins?
    - A. Mitochondria
    - B. Endoplasmic reticulum
    - C. Nucleolus
    - D. Golgi apparatus

Answer: D

11. The door to your house is like the ___ of a cell membrane?
    - A. Phospholipid bilayer
    - B. Gated channel
    - C. Receptor protein
    - D. Recognition protein

Answer: B

12. The phospholipid bilayer of the cell membrane is like a(n):
    - A. Screen door
    - B. Plate glass window
    - C. Hot water heater
    - D. Oven

Answer: A

13. Facilitated diffusion ____ require energy and uses the help of ______
    - A. Does, transport proteins
    - B. Does, cytoplasm
    - C. Does not, transport proteins
    - D. Does not, sodium pumps

Answer: C

14. A semi permeable membrane is stretched across a chamber filled with water. The membrane is only permeable to water. 60 mg of salt is added to the left side of the chamber. Which of the following will happen?
    - A. Water will move toward the right side
    - B. Salt will move toward the right side

C. Water will move toward the left side

D. Salt will move toward the left side

Answer: C

15. The lipid bilayer keeps the inside of the cell membrane:

D. Wet

E. Dry

F. Semi liquid

G. None

Answer: A

16. Which of the following could be found in BOTH the nucleus and the cytoplasm

A. Nucleolus

B. Ribosomes

C. RNA

D. Both RNA & ribosomes

Answer: D

17. Amino acid chains built by the ribosomes then move to the:

A. Golgi apparatus

B. Lysosome

C. Endoplasmic reticulum

D. Mitochondria

Answer: C

18. Which of the following structures has a 9 + 2 arrangement?

A. Flagella

B. Ribosome

C. Mitochondria

D. Golgi apparatus

Answer: A

19. The centriole is most like the:

A. Lysosome

B. Flagella

C. Mitochondria

D. Chromatin

Answer: B

20. Which of the following is composed of a large and a small subunit?

A. Golgi apparatus

B. Endoplasmic reticulum

C. Mitochondria

D. Ribosome

Answer: D

21. A cell that is missing lysosomes would have difficulty doing what?
    - A. Digesting food
    - **B.** Storing energy
    - C. Packaging proteins
    - D. Moving cytoplasm

Answer: A

22. Which of the following cell parts is described as a "fluid mosaic"?
    - A. Chloroplast
    - B. Vacuole
    - C. Cell membrane
    - D. Endoplasmic reticulum

Answer: C

23. Some cells take in large molecules through the process of:
    - A. Protein synthesis
    - B. Endocytosis
    - C. Cytoplasmic streaming
    - D. ATP

Answer: B

24. Which of the following organelles would NOT be found in a plant cell?
    - A. Chloroplast
    - B. DNA
    - C. Food vacuole
    - D. Cell membrane

Answer: C

25. Which of the following organelles is most important in providing energy to the cell?
    - A. Mitochondrion
    - B. Centrosome
    - C. Nucleus
    - D. Peroxisome

Answer: A

26. Name the membrane valves that open and close for potassium efflux and sodium influx.
    - A. Ion channels
    - B. Vacuoles
    - C. Capillaries
    - D. Cytokines

Answer: A

27. What is another name for programmed cell death?
    - A. Necrosis
    - B. Oxidative burst

C. Diapedesis
D. Apoptosis

Answer: D

28. Name the violent membrane blebbing exhibited by a cell undergoing apoptosis.
A. Fission
B. Zeiosis
C. Necrosis
D. Apoptotic cells do not bleb

Answer: B

29. Where are ribosomes produced in a eucaryotic cell?
A. Endoplasmic reticulum
B. Vacuole
C. Centrosome
D. Nucleolus

Answer: D

30. What organelle serves as a primary "packaging" area for molecules that will be distributed throughout the cell?
A. Mitochondrion
B. Vacuole
C. Cytoskeleton
D. Golgi

Answer: C

31. What organelle in higher plant cells contains chlorophyll?
A. Chloroplasts
B. Cytosol
C. Secretory vesicles
D. Nucleus

Answer: A

32. Are vacuoles more prominent in plant or animal cells?
A. Animal
B. Plant
C. Both
D. None

Answer: B

33. The endoplasmic reticulum is an extension of which of these membranes?
A. Cell membrane
B. Outer nuclear membrane
C. Inner nuclear membrane
D. None

Answer: B

34. Which is smallest of these four?
    - A. Bacterium
    - B. Red blood cell
    - C. Virus
    - D. Lymphocyte

Answer: C

Mitosis

35. The process of mitosis ensures that:
    - A. Each new cell is genetically different from its parent
    - B. Each new cell receives the proper number of chromosomes
    - C. Cells will divide at the appropriate time
    - D. DNA is replicated without errors

Answer: B

36. In what stage of mitosis do the chromosomes align on the spindle's equator?
    - A. Telophase
    - B. Metaphase
    - C. Anaphase
    - D. Prophase

Answer: B

37. Which of the following is not part of mitosis
    - A. Prophase
    - B. Metaphase
    - C. Telophase
    - D. Interphase

Answer: D

38. Which of the following is not part of the chromosome?
    - A. Kinetochore
    - B. Chromatid
    - C. Centromere
    - D. Spindle

Answer: D

39. A cell that has 20 chromosomes undergoes mitosis. Which of the following is true?
    - A. Two daughter cells will be created, each have 20 chromosomes
    - B. Two daughter cells will be created, each have 40 chromosomes
    - C. 4 daughter cells will be created, each having 10 chromosomes
    - D. 2 daughter cells will be created, each having 10 chromosomes

Answer: A

40. A spindle forms during which phase?
    - A. G2

B. Interphase
C. Prophase
D. Metaphase

Answer: C

41. Compared to the X chromosome, the Y chromosome is:
A. Much larger
B. Much smaller
C. More twisted
D. Inherited more often

Answer: B

42. Which of the following can be determined from a karyotype?
A. The sex of the individual
B. Whether the individual has Down Syndrome
C. The number of chromosomes present
D. All of above

Answer: D

43. Most cells spend their lives in:
A. Prophase
B. Metaphase
C. Interphase
D. Telophase

Answer: C

44. Cytokinesis begins during which phase?
A. Telophase
B. Synthesis phase
C. Anaphase
D. Metaphase

Answer: A

Meiosis

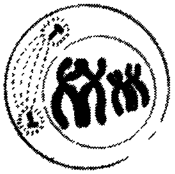
45. Meiosis results in _____
A. 2 haploid daughter cells
B. 4 haploid daughter cells
C. 2 diploid daughter cells
D. 4 diploid daughter cells

Answer: D

46. Which of the following cells undergo meiosis?
A. Sperm cells
B. Liver cells
C. Unicellular organisms
D. All of these

Answer: A

47. The picture depicts what phase of meiosis
    - A. Propase 1
    - B. Prophase 2
    - C. Anaphase 1
    - D. Anaphase 2



Answer: A

48. Crossing-over occurs during:
    - A. Anaphase 1
    - B. Metaphase 1
    - C. Prophase 1
    - D. Prophase 2

Answer: C

49. Meiosis is a type of cell division that produces:
    - A. Zygotes
    - B. Chromosomes
    - C. Dna
    - D. Gametes

Answer: D

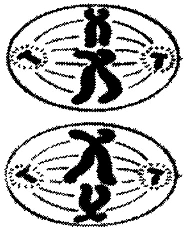
50. Which of the following distinguishes prophase 1 of meiosis from prophase of mitosis?
    - A. Homologous chromosomes pair up
    - B. Spindle forms
    - C. Nuclear membrane breaks down
    - D. Chromosomes become visible

Answer: A

51. A cell with a diploid number of 24 undergoes meiosis, how many chromosomes are in each daughter cell?
    - A. 6
    - B. 12
    - C. 24
    - D. 48

Answer: B

52. The picture depicts what stage of meiosis?
    - A. Prophase 1
    - B. Anaphase 1
    - C. Metaphase 1
    - D. Metaphase 2



Answer: D

53. In what stage of mitosis do the centromeres split and the sister chromatids move apart?
    - A. Telophase
    - B. Metaphase
    - C. Prophase
    - D. Anaphase

Answer: D

54. What is a single cell that results from the fertilization of an egg cell by a sperm cell?
    - A. An embryo
    - B. A fetus
    - C. A haploid cell
    - D. A zygote

Answer: D

55. What is the process by which haploid nuclei are formed from diploid nuclei?
    - A. Meiosis
    - B. Asexual reproduction
    - C. Mitosis
    - D. Binary fission

Answer: A

56. What is the state in which a cell spends most of its life?
    - A. Telophase
    - B. Metaphase
    - C. Prophase
    - D. Interphase

Answer: D

57. How many chromosomes do all of your body cells have?
    - A. 25
    - B. 23
    - C. 48
    - D. 46

Answer: D

58. The cell cycle includes interphase and _________ .
    - A. Respiration
    - B. Meiosis
    - C. Mitosis
    - D. Photosynthesis

Answer: C

59. In what stage of mitosis do the chromosomes align on the spindle's equator?
    - A. Telophase
    - B. Metaphase
    - C. Anaphase
    - D. Prophase

Answer: B

60. Cells having two of each chromosome are called ______ cells.
    - A. Diploid
    - B. Haploid
    - C. Gamete
    - D. Triploid

Answer: A

Indicate whether the sentence or statement is true (T) or false (F).

1. Robert Hooke observed cork cells under a microscope.

Answer: T

2. Anton van Leeuwenhoek concluded that all plants are composed of cells.

Answer: F

3. All living things are composed of many cells.

Answer: F

4. A cell is the smallest unit that can carry on all the processes of life.

Answer: T

5. Inside smaller cells, materials and information can be transported more quickly.

Answer: T

6. Membranes are selectively permeable if they allow only certain substances to diffuse across them.

Answer: T

7. Lysosomes carry on cellular respiration.

Answer: F

8. Microtubules and microfilaments form the cytoskeleton of cells.

Answer: T

9. Colonial organisms differ from single-celled organisms in that each cell cannot support its own existence.

Answer: F

10. The information needed by a cell to direct its activities and to determine its characteristics is contained in molecules of deoxyribonucleic acid (DNA).

Answer: T

11. Gametes are diploid so that when fertilization occurs, the resulting zygote will have the characteristic number of chromosomes for that species.

Answer: F

12. A karyotype is a type of gene.

Answer: F

13. Cell division in bacteria and eukaryotes takes place in precisely the same manner.

Answer: F

14. Cells spend most of their lifetime in interphase.

Answer: T

15. After the replication of a cell's chromatids, there are twice as many centromeres as there are chromosomes.

Answer: F

16. Asexual reproduction occurs by mitosis.

Answer: T

17. During telophase, a nuclear envelope surrounds each new set of chromosomes.

Answer: T

18. Chromatids separate from each other during telophase.

Answer: F

19. After mitosis and cytokinesis, each new cell has a complete set of the original cell's chromosomes.

Answer: T

20. Mitosis is a cell division process that occurs in somatic cells, however, meiosis is a cell division process in specialized tissues of ovaries and testes which result in the production of sex cells.

Answer: T

Complete each sentence or statement.

1. The statement that "cells are produced only from existing cells" is part of the ____________________.

Answer: cell theory

2. The ratio of surface area to volume puts limitations on a cell's ____________________.

Answer: size

3. Eukaryotic cells are much larger and have more specialized functions than prokaryotic cells because they contain ____________________ which take up space and carry out specialized activities.

Answer: organelles

4. A cell with a well-defined nucleus surrounded by a nuclear membrane is called a(n) ____________________ cell.

Answer: eukaryotic

5. A cell membrane is said to be ____________________ permeable because it allows the passage of some solutes and not others.

Answer: selectively

6. ____________________ molecules have "heads" and "tails" and are found in the cell membrane.

Answer: Lipid

7. Scientists have discovered that cells contain smaller specialized structures known as ____________________.

Answer: organelles

8. The spherical organelles that are the site of protein synthesis in a cell are the ____________________.

Answer: ribosomes

9. The meshlike network of protein fibers that supports the shape of the cell is called the ____________________.

Answer: cytoskeleton

10. The fluid portion of the cytoplasm is called the ____________________.

Answer: cytosol

11. Photosynthesis takes place in the ____________________ of plant cells.

Answer: chloroplasts

12. Following replication of its DNA, each chromosome contains two ____________________, which are attached to each other by a centromere.

Answer: chromatids

13. Chromosomes that are not involved in sex determination are called ____________________.

Answer: autosomes

14. A picture of a cell's chromosomes is called a ____________________.

Answer: karyotype

15. The sequence of events that occurs in a cell from one mitotic division to the next is called the ____________________.

Answer: cell cycle

16. "Cables" made of microtubules that extend from the poles of a cell to the centromeres during cell division are called ____________________.

Answer: spindle fibers

17. In mitosis, anaphase follows ____________________.

Answer: metaphase

18. The stage of meiosis during which homologues line up along the equator of the cell is called ____________________.

Answer: metaphase I

19. ______________are a thread-like, gene-carrying bodies in the cell nucleus. They are composed primarily of DNA and protein. They are visible only under magnification during certain stages of cell division.

Answer: Chromosomes

20. ____________cells in the body that are not directly involved with reproduction.

Answer: Somatic

Water

Identify the letter of the choice that best completes the statement or answers the question.

1. Normal metabolic activity can occur only when cells are at least ____% H2O.

   A. 23 B. 45 C. 53 D. 65

Answer: D

2. Which of the following statements about water is false?
   A. Water can serve as both a donor and an acceptor in hydrogen bond formation.
   B. Water has an unusually high dielectric constant.
   C. Pure liquid water consists of H_2O molecules in a highly ordered three- dimensional network of hydrogen bonds.
   D. The maximum density of water is found in the liquid state.

Answer: C

3. What property of water best explains its excellent solvent abilities for ionic substances?
   A. Its anomalously low boiling point
   B. Its highly polar nature
   C. Its low dielectric constant
   D. The 109° H-O-H bond angle

Answer: B

4. The average lifetime of hydrogen bonds between water molecules is about how long?

   A. 10 picoseconds A. 10 nanoseconds C. 10 microseconds D. 10 seconds

Answer: A

5. The constant Kw, the ion product of water, can be written as:
   A. $Keq = 10^{14} = [H+][OH-]$ C. $Keq = 10^{-14} = [H+][OH-]$
   B. $Keq = 10^{-12} = [H+][OH-]$ D. $Keq = 10^{-7} = [H+][OH-]$

Answer: C

6. If the pH of a solution is 7, then the concentration of hydrogen ions in that solution is:

   A. 10^{14} B. 10^{-14} C. 10^{7} D. 10^{-7}

Answer: D

7. pH = pKa + log10 [A-]/[HA]:
   A. is known as the Henderson-Hasselbalch equation
   B. provides a general solution to the quantitative treatment of acid-base equilibria

C. contains [A-], which represents a weak acid's conjugate base
D. all of the above

Answer: D

8. Phosphoric acid, H_3PO_4:
A. is a polyprotic acid B. requires two equivalents of OH- to neutralize it
C. is a strong acid D. has a pKa of 2.38

Answer: A

9. Phosphoric acid, H3PO4, serves to buffer the intracellular fluid of cells at physiological pH because:
A. it is a naturally occurring amino acid
B. phosphate is only found in an organic form in biological molecules
C. its pK2 lies near the physiological pH value of the cell
D. its pK1 lies near the physiological pH value of the cell

Answer: C

10. Which of the following statements is true of histidine?
A. The concentration of free histidine is low in cells.
B. Protein-bound and dipeptide histidine may be the dominant buffering system in some cells.
C. The pKa for dissociation of the imidazole hydrogen of histidine is 6.04.
D. All of the above are true.

Answer: D

11. The bicarbonate buffer system of blood plasma works well because:
A. the concentration of H2CO3 is maintained relatively constant
B. the concentration of H2PO4 is maintained relatively constant
C. Both are true
D. none of above true

Answer: A

12. Which of the following is true about buffer systems?
A. They are typically made up of a strong acid and a strong base mixed together.
B. The pH will vary greatly in the region of the titration curve where [HA] = [A-].
C. They will have an area on their titration curve where the greatest buffering capacity exists.
D. They will vary greatly in pH as acid or base is added.

Answer: C

13. Hydrogen bonds can form between:
A. hydrogen atom covalently bonded to an electronegative atom and a second electronegative atom

B. a nitrogen atom covalently bonded to an electronegative atom and a nitrogen atom on another molecule.

C. a hydrogen atom ionically bound to an electropositive atom and a second electronegative atom

D. a hydrogen covalently coupled to an electropositive atom and a second electronegative atom

Answer: A

14. Which of the following has the highest affinity for protons?

A. Lactic acid - pKa = 3.86
C. Pyruvic acid - pKa = 2.50
B. Acetic acid - pKa = 4.76
D. Cysteine acid - pKa = 1.71

Answer: B

15. A buffer of HPO_4^{2-} /$H_2PO_4^-$ has a pH of 7.4. What is the ratio of HPO_4^{2-} /$H_2PO_4^-$ in this buffer? The pK of the HPO_4^{2-} /$H_2PO_4^-$ couple is 7.2.

A. 1.6 B. 0.62 C. 2 x 10-3 D. 7.0

Answer: A

16 The pH of a solution with a hydroxyl ion concentration of 4 x 10^{-3} is:

A. 11.6 B. 3.6 C. 2.4 D. 10.4

Answer: A

17 Which of the following is a weak acid?

A. Nitric acid
C. Phosphoric acid
B. Sulfuric acid
D. Hydrochloric acid

Answer: C

18. The cytoskeleton of a cell:

A. functions in the intracellular digestion of materials entering the cell

B. is the powerplant of the cell where fats, proteins, and carbohydrates are oxidized to provide energy

C. determines the shape of the cell and gives it the ability to move

D. is the selectively permeable outer boundary of the cell

Answer: C

19. In the formation of biomolecules, carbon forms stable, covalent bonds by electron sharing. Carbon can form as many as ____ such bonds.

A. three B. four C. five D. six

Answer: B

20. The four atoms that comprise more than 99% of the atoms in the human body are:

A. hydrogen, carbon, oxygen, nitrogen

B. carbon, oxygen, phosphorus, sulfur

C. nitrogen, oxygen, calcium, iron

D. carbon, hydrogen, sodium, potassium

Answer: A

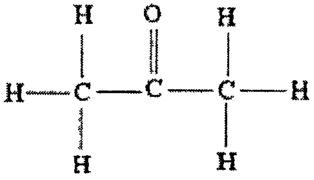
Introduction to Biochemistry

Identify the letter of the choice that best completes the statement or answers the question.

1. All of the following are carbohydrates except:
A. Starch, B. Glycogen, C. Chitin D. Cholesterol

Answer: D

2. The structure contains which functional group
A. Aldehyde
B. Ketone
C. Amino
D. Carboxyl



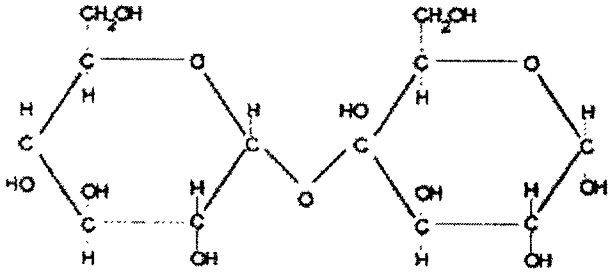
Acetone

Answer: B

3. Fatty acids that are unsaturated have:
A. An amino group B. A double bond
C. An excess of protons D. A carboxyl group

Answer: B

4. The structure below is a:



A. Monosaccharide B. Disaccharide
C. Lipid D. Polymer

Answer: B

5. Which of the following can have a quaternary structure?
A. Fatty acid B. Protein
C. Polysaccharide D. DNA

Answer: B

6. An organic compound is one that:
A. Contains carbon B. Is slightly acidic
C. Forms long chains D. Is soluble in water

Answer: A

7. Which of the following elements is the LEAST abundant in living organisms?
A. Oxygen B. Nitrogen

C. Phosphorous D. Sodium

Answer: D

8. Which of the following is used in PET scans?
A. Uranium B. Ions
C. Isotopes D. Steroids

Answer: C

9. Carbon can form ___ separate bonds with other elements?
A. 1 B. 2 C. 3 D. 4

Answer: D

10. The cohesion of water is caused by:
A. Ionic bonds B. Hydrophobic compounds
C. Hydrogen bonds D. Covalent bonds

Answer: C

11. Biology is the study of
A. Minerals. B. Life C. The weather. D. Energy.

Answer: B

12. Homeostasis means
A. A change over long periods of time.
B. Keeping things the same.
C. Rapid change.
D. The same thing as evolution.

Answer: B

13. Which of the following is a means by which heterotrophs can obtain energy?
A. Using water, carbon dioxide, and energy from the sun to produce sugars
B. Using water and carbon dioxide to produce energy-rich compounds
C. Consuming autotrophs
D. Consuming simple chemicals from the environment and using them to assemble complex chemicals and structures needed by the organism

Answer: D

14. Which of the following is not necessarily a distinct property of living things?
A. Homeostasis C. Complexity
B. Metabolism D. Reproduction

Answer: C

15. All organisms are composed of
A. Diatoms C. Cells
B. Cellulose D. None of the above

Answer: C

Chapter 2

Carbohydrates

Nature and Nomenclature

Carbohydrates are a large family of naturally occurring compounds including sugars, starches, and cellulose, as well as materials found in bacterial cell walls and insect exoskeletons. Carbohydrates, in general, contain a C-C skeletal monomers bearing C=O and OH (and sometimes NH_2) functional groups. These skeletal monomers form polymers through a C-O-C linkage. Carbohydrates can also be called saccharides, a general word derived from the Latin word for sugar. The nomenclature of sugars uses the suffix, or word name ending, **-ose** to indicate a sugar e.g., : gluc*ose*.

The carbohydrates comprise one of the major groups of naturally occurring biomolecules. This is mainly because; the light energy from the sun is converted into chemical energy by plants through primary production and is transferred to sugars and carbohydrate derivatives. The dry substance of plants is composed of 50-80% of carbohydrates. The structural material in plants is mainly cellulose and related hemicelluloses. Starch is the important form of storage polysaccharide in plants. Pectins and sugars such as sucrose and glucose are also plant constituents. Many non-carbohydrate organic molecules are found conjugated with sugars in the form of glycosides. The carbohydrates in animals are mostly found in combination with proteins as glycoproteins, as well as other compounds. The storage form of carbohydrates, glycogen, found in liver and muscles, the blood group substances, mucins, ground substance between cells in the form of mucopolysaccharides are few examples of carbohydrates playing important roles in animals. Chitin found in the exo-skeleton of lower animals, is a polymer of N-acetyl glucosamine. Carbohydrates are also universally found in other polymeric substances. For example, fats are fatty acid esters of a sugar alcohol, glycerol. Ribose and deoxyribose are constituent of nucleic acids. Moreover, in all living forms, the energy needed for mechanical work and chemical reactions are derived from carbohydrates. Adenosine triphosphate and related substances that contain ribose as a constituent are key substances in energy storage and transfer. The carbon skeletons of almost all organic molecules are derived from carbohydrates. Besides, the carbohydrates are the basic raw material of many important industries including sugar and sugar products, starch products, paper and wood pulp, textiles, plastics, food processing and fermentation.

Classification of Carbohydrates

Carbohydrates are classified into three major groups :
Monosaccharides, Oligosaccharides and Polysaccharides

Monosaccharides (Simple Sugars)

- Simplest form that cannot be hydrolyzed further into smaller units.
- Low molecular weight carbohydrates and cannot be hydrolysed further.
- Crystalline, soluble in water, and sweet in taste.
- Classified into triose, tetrose, pentose, hexose and heptose depending upon the number of carbon atoms. They may be either aldoses or ketoses depending upon whether they contain a (potential) free aldehyde (OH) or ketone (C=O) group, respectively.
- All monosaccharides are reducing in nature.

Oligosaccharides

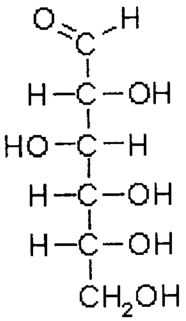
- Contain 2-10 monosaccharides joined by glycosidic bonds. Low molecular weight carbohydrates which can be hydrolysed by enzymes or acids to yield monosaccharides.
- Powdery or crystalline, soluble in water and sweet in taste.
- Classified into disaccharide, trisaccharide, tetrasaccharide and pentasaccharide depending upon the number of monosaccharides they contain.
- Some of them are reducing and some of them are non reducing in nature

Polysaccharides (Glycans)

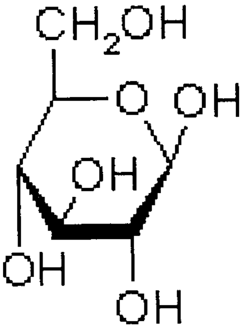
- Contain many monosaccharides joined by glycosidic bonds. They can be hydrolysed by enzymes or acids.
- Insoluble in water, tasteless, linear or branched.
- Classified into homoglycans and heteroglycans depending upon the kind of monosaccharides present. Depending upon the function, they are classified as storage and structural polysaccharides.
- Non reducing in nature.

Monosaccharides

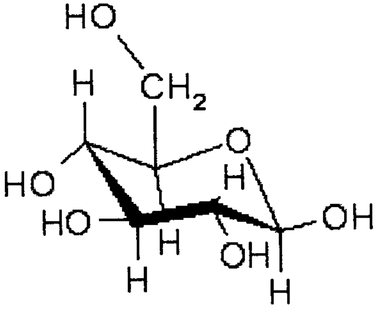
Monosaccharides are the building blocks of larger sugars, and their biochemistry is possibly the most complex and confusing of all the biochemicals. Here is a brief list of the key terms in monosaccharide nomenclature for glucose:



D-Glucose,
Fischer projection



β-D-glucopyranose,
Haworth projection



β-D-glucopyranose,
cyclohexane projection

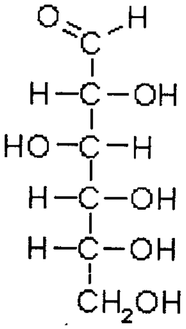
- Number of carbons: six, hence hexose.
- Numbering of carbons: C1 is the carbonyl carbon, C6 the farthest carbon from this.
- Type of carbonyl compound: terminal CHO (aldehyde) hence aldose.
- D/L descriptor: the OH is to the right on the farthest chiral carbon from the CHO group (*i.e.,* C5) in the Fischer projection, and in the Haworth projection, hence D-glucose.
- Names of sugars: the three intermediate chiral carbons (C2, C3, C4) indicate that this is glucose.
- Ring forms: six membered ring with anomeric OH (C1) on same side as CH_2OH group (C5 and C6), hence α-pyranose.

The number of carbons in a sugar determines which size group of sugars it belongs to:

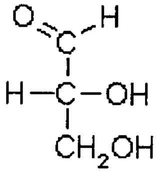
- 3 Carbons : triose - e.g., Glyceraldehydes, Dihidroxiacetone
- 4 Carbons : tetrose - e.g., Erythrose, Erythrulose, Threose
- 5 Carbons : pentose - e.g., Ribose, Ribulose, Xylose, Xylulose
- 6 Carbons : hexose - e.g., Glucose, Galactose, Fructose, Mannose, Sorbose
- 7 Carbons : heptulose - e,g., Sedoheptulose

Open chain monosaccharides (like the Fischer projection above) have n-2 chiral carbons, so 2^{n-2} isomers (assuming no internal symmetry). Note that sugars can also react internally to produce a variety of rings forms: this introduces an extra chiral centre, as you can see in the Haworth projection above.

These are a hexose and a triose.

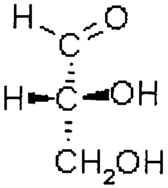


Glucose



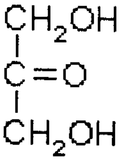
Glyceraldehyde

The number in the chain was given from the CHO group at the top down to the CH_2OH group at the base (i.e., number from the end nearest the carbonyl group). This way of drawing monosaccharides is called a Fischer projection: imagine the vertical bonds curling away from you and the horizontal bonds sticking out at you, like the back of a stegosaurus.

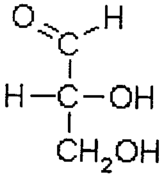


Interpreting Fischer projections.

The type of carbonyl group present in a sugar is an important determinant of its chemical properties. Sugars with a terminal CHO group are chemically aldehydes, and are termed *aldoses e.g.,* glyceraldehyde. These sugars are 'reducing sugars': they reduce the blue Cu^{2+} in Benedict's solution to a red precipitate of Cu^{+}. This is the test for reducing sugars so beloved of A-level syllabi. Sugars with an internal CO group, R-CO-R are ketones, and are called *ketoses e.g.,* dihydroxyacetone (DHA). Although ketones are not generally reducing agents, the OH attached to the next carbon along is capable of reducing Benedict's reagent, hence all monosaccharides are 'reducing sugars'.



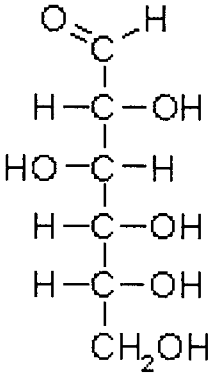
Dihydroxyacetone



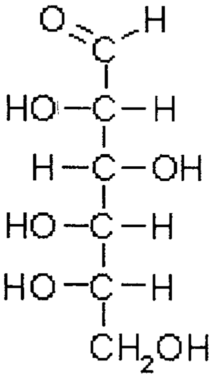
D-glyceraldehyde

Sugars have a somewhat arcane nomenclature. So far it has been mostly logical, but from now on, it's largely arbitrary decisions made fifty years ago or more. Apologies for this.

Sugars contain many chiral centres. Naming these chirals centres should be easy (just use R or S descriptors), but in fact, there are three systems for different bits of the sugar molecule. The first descriptor is used to name the *chiral carbon farthest from the carbonyl group.* This is called the D/L descriptor. If you draw sugar in the Fischer projection (as above), an OH to the right on the farthest chiral carbon is the D-form, and an OH to the left is the L-form. D-glyceraldehyde appears above. Note that DHA is nonchiral (it's about the only sugar that is!). The two forms of glucose are below, note that they differ at every chiral centre:

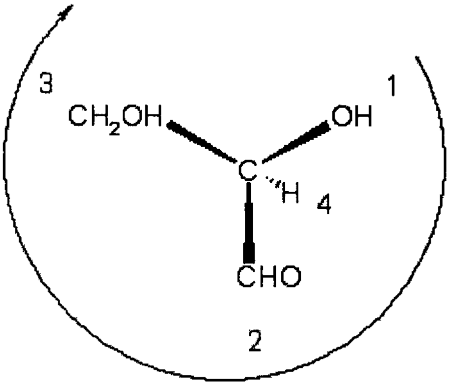


D-glucose



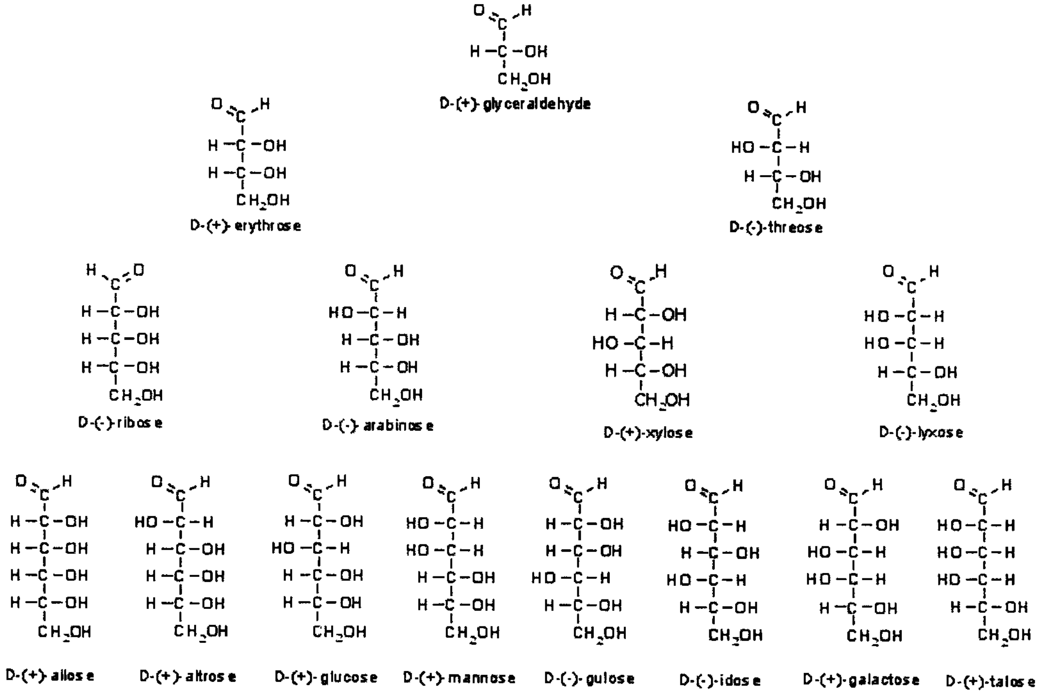
L-glucose

The D-isomer of glyceraldehyde also happens to be the R isomer using CIP rules, as you can see from the following diagram.


3
1
CH₂OH
OH
C
H
4
CHO
2

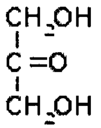
It also happens to be the (+) isomer from polarimetry. This is easy to remember (R, D and +), but only by fluke! The remaining chiral centres in an open chain monosaccharide get 'proper' names. These are the D-forms of the common monosaccharides. Note that they differ only at carbons 2, 3 and 4.

Aldoses

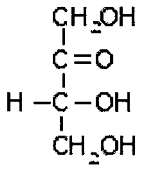

D-(+)-glyceraldehyde
D-(+)-erythrose
D-(-)-threose
D-(-)-ribose
D-(-)-arabinose
D-(+)-xylose
D-(-)-lyxose
D-(+)-allose
D-(+)-altrose
D-(+)-glucose
D-(+)-mannose
D-(-)-gulose
D-(-)-idose
D-(+)-galactose
D-(+)-talose

D-galactose and D-glucose differ at only one chiral centre: such a relationship makes them *epimers*.

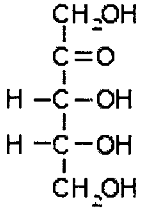
Ketoses



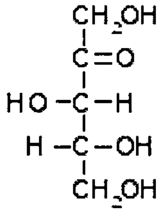
dihydroxyacetone



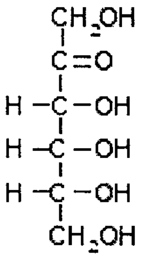
D-(-)-erythrulose



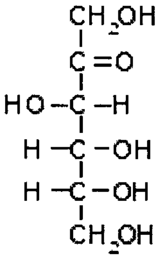
D-(+)-ribulose



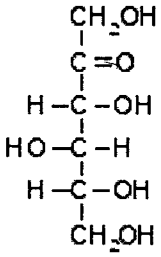
D-(+)-xylulose



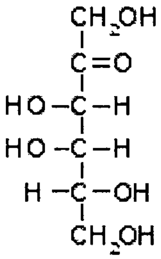
D-(+)-psicose



D-(-)-fructose

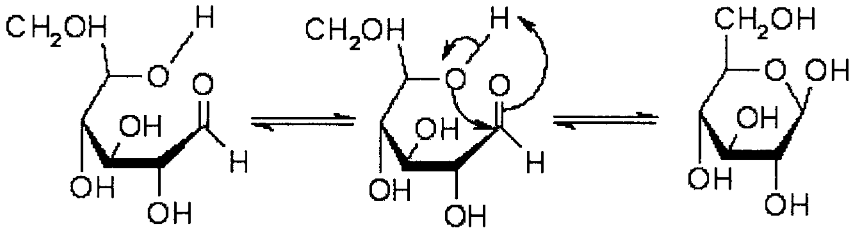


D-(+)-sorbose

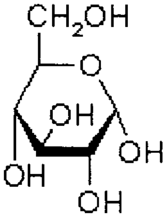


D-(-)-tagatose

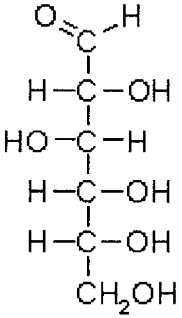
The third descriptor for monosaccharides only comes into play when sugars internally react to form rings. Monosaccharides react internally by nucleophilic attack of the carbonyl group by a OH group, to form a cyclical hemiacetal:.



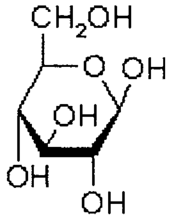
Two chiral ring forms are possible, which introduces a new chiral center, so we now have (maximally) 2^{n-1} isomers. These diastereomers are called the α and β *anomers*. The α anomer has it's 'new' OH on the opposite side of the molecule to the CH_2OH group. The β anomer has it on the same side, as you can see below.



α-D-glucopyranose,
Haworth projection



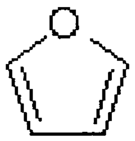
Open chain glucose,
Fischer projection



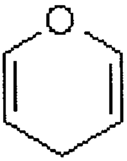
β-D-glucopyranose,
Haworth projection

For glucose solutions, typically, the ratios of forms are 36% α, 63% β, <1% open chain. When you dissolve glucose in water, the slow conversion of the solid equilibrium mixture to the aqueous equilibrium mixture means that the rotation glucose causes to polarised light decreases (becomes less positive) over time. This is called ***mutarotation.***

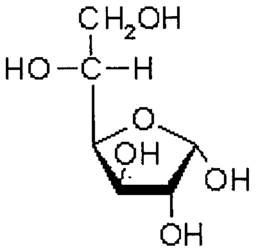
A final complication for the ring forms is that the rings of hexoses and pentoses can be either 5 or 6 membered (including the heterocyclic oxygen atom), depending on which carbon (usually 4 or 5) the CO group is attacked by. Five membered rings are called furanoses, after the chemical furan. Six membered rings are called pyranoses, after pyran. These are glucopyranose and glucofuranose.



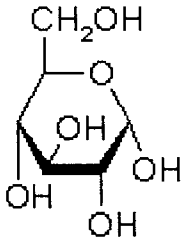
Furan



Pyran

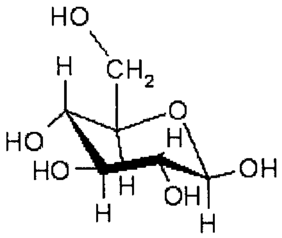


α-D-glucofuranose

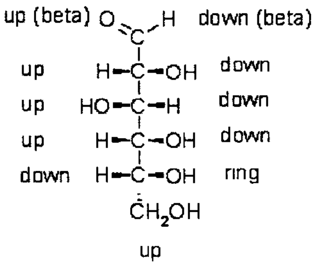


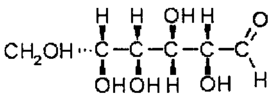
α-D-glucopyranose

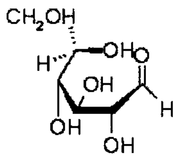
The Haworth notation has been used above to show the relative positions of the OH groups in the ring form. Haworth rotation is a simple and tidy way of representing ring forms, and is fairly self explanatory. Sugars actually take up cyclohexane shapes in the pyranose form, as shown below for glucose, but Haworth notation is considerably easier to interpret.

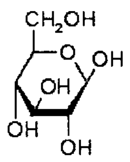


To convert between Haworth and Fischer projections is a little tricky. Remember that the Fischer projection curves away from you, with the OH and H groups sticking out like the plates on a stegosaur's back. If you turn the Fischer projection clockwise through 90° in your mind's eye, you will see that for carbons 2, 3, and 4, if the Fischer OH goes right, the Haworth OH goes down. Carbon 1 is the anomeric carbon, and doesn't exist in the open chain form, so we're left with carbon 5 to understand. You need to realise there is free rotation of the H, OH and CH2OH groups around carbon-5, hence you can twirl the three groups round so that the CH2OH is pointing up as you curl the chain round into a ring.









Occurrence

- Intermediary metabolities in glucose metabolism.
- Ribose is a constituent of nucleic acid.
- Occurs in polysaccharides.
- Gum arabic, cherry gums, wood gums, proteoglycans.
- Fruit juices and cane sugar .
- Lactose, constituent of lipids.
- Plant mannosans and glycoproteins.
- Intermediate in carbohydrate metabolism.

Derived Sugars / Sugar Derivatives

The important functional groups present in monosaccharides are hydroxyl and carbonyl groups. The hydroxyl group forms esters, usually with phosphoric acid or is replaced by a hydrogen or amino group. The carbonyl group undergoes reduction or oxidation to produce number of derived monosaccharides.

Deoxy Sugars

In sugars, the hydroxyl group is replaced by a hydrogen to produce deoxy sugars (devoid of oxygen). The important deoxy sugar is 2-deoxy ribose that occurs in deoxy ribonucleic acid. Other important deoxy sugars are L-fucose and L. rhamnose.

The substitution of the hydroxyl group at C-6 of L. galactose or L.mannose with hydrogen produces fucose or rhamnose respectively. L-fucose occurs in the cell wall polysaccharides namely hemicelluloses and L-rhamnose occurs in pectic polysaccharides namely rhamnogalacturonan. These deoxy sugars are also found in the complex oligosaccharide components of glycoproteins and glycolipids.

Amino Sugars

The hydroxyl group, usually at C-2, is replaced by an amino group to produce amino sugars such as glucosamine, galactosamine and mannosamine. The amino group may be condensed with acetic acid to produce N-acetyl amino sugars, for example, N-acetyl glucosamine. This glucosamine derivative is important constituent of many structural polymers (chitin, bacterial cell wall polysaccharides etc.).

Polyols (alditols)

Both aldoses and ketoses are reduced to polyhydric alcohols (polyols) when treated with enzymes, sodium amalgam, and hydrogen under high pressure with catalyst or sodium borohydride. Each aldose yields the corresponding alcohol upon reduction while a ketose forms two alcohols because of the appearance of a new asymmetric carbon atom in the process. By this reduction process, the following sugars give rise to their respective alcohols under specified conditions.

Glucose → Sorbitol
Fructose → Sorbitol and mannitol
Mannose → Mannitol
Glyceraldehyde → Glycerol
Erythrose → Erythritol
Ribose → Ribitol
Galactose → Dulcitol

Polyols occur in many plant products. Sorbitol was first isolated from the berries of mountain ash *(Sorbus aucuparia)*. Commercially sorbitol is manufactured by the hydrogenation of glucose. Mannitol occurs in many terrestrial and marine plants. Potential food applications of polyols include confectionery products, bakery products, deserts, jams and marmalade. Sorbitol is an excellent moisture conditioner and is used in pharmaceutical preparations such as elixirs and syrups. Sorbitol, as a humectant in creams and lotions helps to stabilize the water content, providing better moisture control. The use of sorbitol or xylitol in toothpaste and mouthwashes is highly desirable.

Oxidation Products

When aldoses are oxidized under proper conditions with different types of oxidizing agents, three types of acids are produced, namely aldonic acids, uronic acids and aldaric acids or saccharic acids.

Aldonic Acids

Oxidation of an aldose with bromine water at neutral pH converts the aldehyde group to a carboxyl group. Hydrobromous acid formed by the reaction of water with bromine acts as an oxidizing agent. Ketoses are not readily oxidized by bromine water. Aldoses are not only oxidized by bromine water but also by the alkaline iodine solution.

$$I_2 + 2\,NaOH \longrightarrow NaOI + NaI + H_2O$$

$$\text{Glucose} + NaOI \longrightarrow \text{gluconic acid}$$

Uronic Acids

When aldoses are oxidised with hydrogen peroxide (H_2O_2) uronic acids are formed. In this reaction only primary alcohol group is oxidized to carboxyl group, whereas the aldehyde group remains unchanged. Uronic acids are constituents of pectic polysaccharides.

Aldaric or Saccharic Acid

When aldoses are oxidised with nitric acid, saccharic acids are formed. Both aldehyde and primary alcohol groups are oxidised to carboxyl groups. Glucose on oxidation with nitric acid produces glucaric or glucosaccharic acid. The aldaric acid produced from galactose is called as mucic acid.

Thus, various types of Derived Sugars and their Occurance are summarized in Table 2.1.

Table 2.1 : Some Important Derived Sugars and their Occurance.

Type of Derived Sugars	No. of Carbon Atoms	Name of Derived Sugars	Occurance
Deoxysugar	5	2-Deoxy ribose	DNA
	6	L-Rhamnose	Component of cell wall
Aminosugar	6	D-Glucosamine	A major component of polysaccharide found in insects and crustaceans (chitin)
Polyol	6	Sorbitol	Berries
	6	Mannitol	Commercially prepared from mannose and fructose
Aldonic acid	6	Gluconic acid	-
Uronic acid	6	Glucuronic acid	Constituent of chondroitin sulfate
	6	Galacturonic acid	Constituent of pectin
Aldaric acid (Saccharic acid)	6	Glucaric acid	Oxidation product of glucose
	6	Mucic acid	Oxidation product of galactose

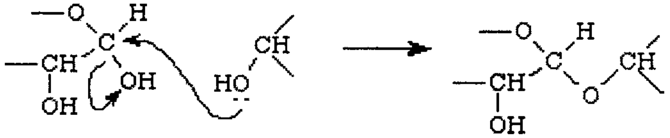
Oligosaccharides

The general term oligosaccharide, is often used for carbohydrates that contain from four to ten monosaccharide units. We will look at some of the common disaccharides, examine their bonding patterns and properties and then turn to some common polysaccharides.

The oligosaccharides commonly encountered in nature belong to disaccharides. The physiologically important disaccharides are maltose, lactose, trehalose and sucrose. Disaccharides consist of two monosaccharides joined covalently by an O-glycosidic bond. The hydroxyl group formed as a result of hemiacetal formation is highly reactive when compared to other hydroxyl groups. This hydroxyl group present in one monosaccharide reacts with any one of the hydroxyl groups attached to C-1, C-2, C-3, C-4, or C-6 of another monosaccharide to produce 1→1, 1→2, 1→3, 1→4, and 1→6 linked disaccharides. When only one anomeric carbon is involved in glycosidic bond formation, reducing disaccharides are formed. If both anomeric carbon atoms of monosaccharides are involved in glycosidic bond formation that results in the formation of a non-reducing disaccharides such as trehalose (aldosyl-aldosyl disaccharide) or sucrose (aldosyl-ketosyl disaccharide)'. In the case of reducing disaccharides, one end of the molecule having free anomeric carbon is called reducing end and the other end, where the anomeric carbon is involved in glycosidic bond, is called as non-reducing end.

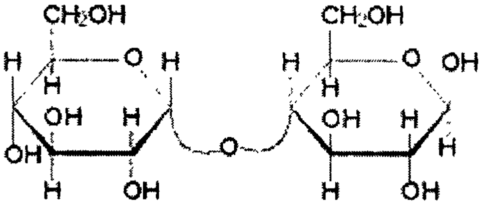
Disaccharides (Table 2.2)

Glycosidic bonds - link sugars via acetal bonds

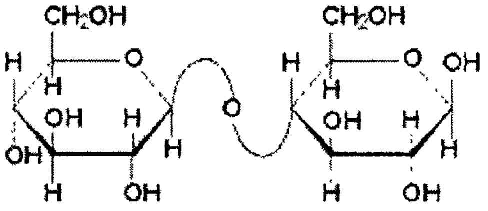


There are four common disaccharides :

- **Maltose** [α-D-Glucopyranosyl-(1,4)-α-D-glucopyranose]
- **Cellobiose** [β-D-Glucopyranosyl-(1,4)-β-D-glucopyranose]



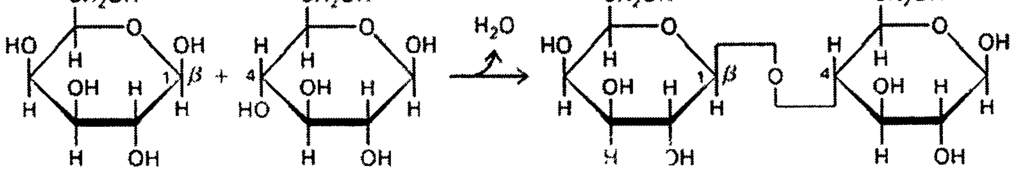
Maltose



Cellobiose

Both maltose and cellobiose are composed of two D-glucose molecules linked together, the only difference between the two disaccharide is the nature of the 1, 4 glucosidic linkage.

- **Lactose** [β-D-Galactopyranosyl-(1,4)-β-D-glucopyranose] –



Galactose **Glucose** **Lactose**

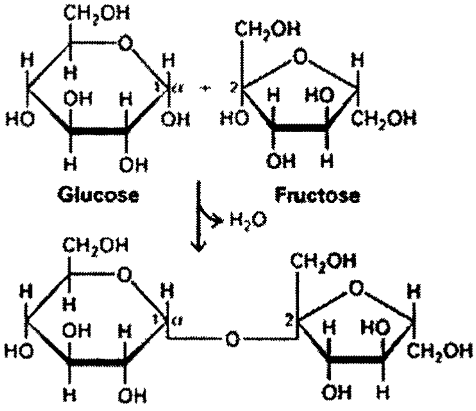
The major sugar found in human and cow's milk (4 to 8% lactose by weight).

In humans, the enzyme in the small intestine responsible for the hydrolysis of the β (1, 4) glycosidic bond is called lactase. This enzyme is not present in all persons, particularly those whose ancestors did not rely upon cow's milk (and milk products) as a food source.

An exception for mammals is the ability of nursing animals to digest lactose. This ability is generaly lost at the age of weaning, at which time the animal becomes lactose intolerant.

A deficiency in the enzyme lactase give rise to: lactose intolerance, and this disorder occurs most commonly among Afro-Americans, Asians, Native Americans and Hispanics.

- **Sucrose** [α-D-Glucopyranosyl-(1,2)-β-D-fructofuranoside]



Sucrose

The most common sugar in our diet is **sucrose**, which is a **disaccharide**.

It is composed of two **monosaccharides** a-D-glucose (α -D-glucopyranose) and b -D-fructose (β -D-fructofuranose).

Sucrose is unusual in that both monosaccharides are linked as acetals

Because both of the potential carbonyl groups in sucrose are tied up in acetals, sucrose shows none of the typical reactions of carbonyl compounds. It will not show positive Tollens test, cannot mutarotate, etc.

Sucrose can also be called: α-D-glucopyranosyl-β-D-fructofuranoside or β-D-fructofuranosyl-α -D-glucopyranoside.

Sucrose is an optically active molecule, [a]D = +66° but its optical activity is quite different from the sum of the optical activities of the two simple sugars, glucose and fructose. In fact an equimolar solution of glucose and fructose rotates light in the opposite direction to the equivalent solution of sucrose. Glucose also rotates the plane of polarization to the right, so that it was once known as dextrose: [a]D = +52.5° for the equilibrium mixture of a and b. By contrast fructose rotates the plane of polarization to the left, so that it was once known as levulose: [a]D = - 92.4° .

The disachharides - maltose, lactose and cellobiose are reducing sugars, that is they have "free" aldehyde groups, whereas sucrose has both carbonyl groups tied up in the relatively stable glycosidic bond. In general, the -glycosidic bond is easily cleaved (it is less stable chemically and organisms have enzymes to cleave it) whereas the beta-glycosidic bond is very difficult to break down.

Thus for cellobiose, and more importantly, **cellulose** which is also linked by β-bonds, essentially only bacteria can digest this bond through cellulase enzyme.

So animals can't digest cellulose! Yes, they can digest through bacterial activity in their digestive system. Cows for instance are basically walking fermentation tanks.

Table 2.2 : Composition, Sources and Properties of Common Disaccharides

Disaccharides	Constituent Monosaccharides	Linkage	Source	Properties
Reducing Disaccharides				
Maltose	α-D-glucose + α-D-glucose	α(1→4)	Germinating cereal and malt	Forms osazone with phenylhydrazine. Fermentable by enzyme maltase present in yeast. Hydrolysed to two molecules of D-glucose. Undergoes mutarotation.
Lactose	β-D-galactose + α-D-glucose	β(1 →4)	Milk. In trace amounts it can be seen in urine during pregnancy.	It shows reactions of reducing sugars including mutarotation. Decomposed by alkali. Not fermentable by yeast. Hydrolysed to one molecule of galactose and one molecule of glucose by acids and the enzyme lactase.

Non Reducing Disaccharides				
Sucrose	α-D-glucose + β-D-fructose	α,β(1→2)	Sugar beet, sugar cane, sorghum and carrot roots.	Fermentable. Hydrolysed by dilute acids or enzyme invertase (sucrase) to one molecule of glucose and one molecule of fructose. Relatively stable to reaction with alkali
Trehalose	α-D-glucose + α-D-glucose	α,α(1→1)	Fungi and yeast. It is stored as a reserve food supply in insect's hemolymph.	It is hydrolysable by acids to glucose with difficulty. Not hydrolysed by enzymes.

Polysaccharides

Polysaccharides are glycosides between sugars. The name given to the polysaccharide is dependent on the size of the molecule:

- 1 sugar unit: monosaccharide
- 2 sugar units: disaccharide
- 3 sugar units: trisaccharide
- 3-several sugar units: oligosaccharide
- 100+ sugar units: polysaccharide

Polysachharides are two types - homo and heteropolysaccharides. Homopolysaccharides have a single type of residue. Most common polysaccharides contain glucose which is used for energy (food), storage (starches and glycogen) and structure (cellulose).

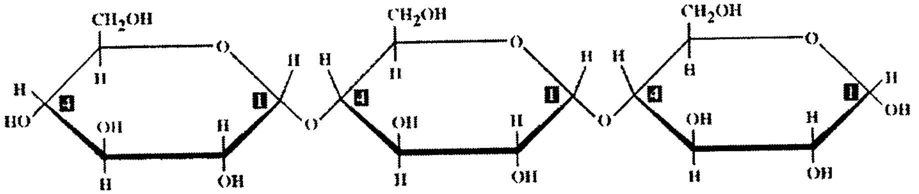
Storage Polysaccharides

Starch (energy storage in plants). Two kinds

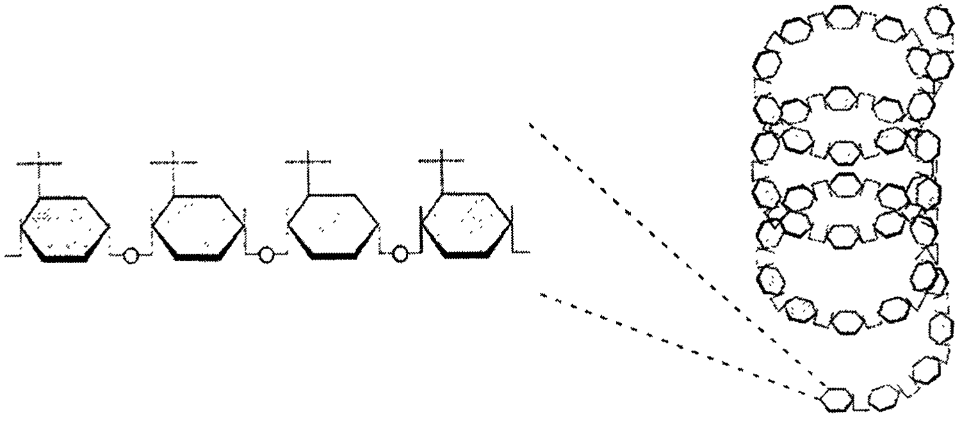
- ***Amylose***: linear, α-1,4 glycosidic links. MW: 4,000-150,000. Deep blue color with iodine due to coiled complex enclosing iodine-colour is lost with heating, returns when cooled.

- ***Amylopectin***: branched every 30 or 50 units - linear α-1,4 chain of 30 glu residues then α-1,6 branch point. Of course get branches on branches as well. MW: ->500,000. Broken down by -amylase (pancreas and salivary glands; random cleavage of α-1,4 links) to give glucose and maltose; or beta-amylase (plants; hydrolyses from reducing ends to give maltose). When either of these enzymes attack amylopectin, they are blocked when they reach or are near a branch, thus end up with a "limit dextrin."

α 1-4 Bonds Between 3 Molecules of Glucose



Starches are carbohydrates in which 300 to 1000 glucose units join together. It is a polysaccharide which plants use to store energy for later use. Starch forms in grains with an insoluble outer layer which remain in the cell where it is formed until the energy is needed. Then it can be broken down into soluble glucose units. Starches are smaller than cellulose units, and can be more readily used for energy. In animals, the equivalent of starches is glycogen, which can be stored in the muscles or in the liver for later use.

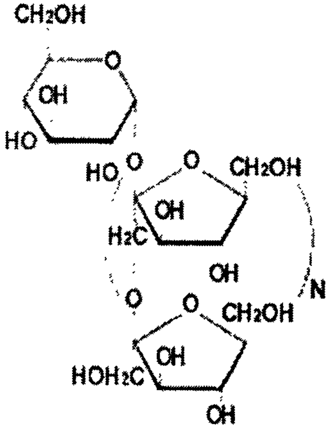


Starch

Foods such as potatoes, rice, corn and wheat contain starch granules which are important energy sources for humans. The human digestive process breaks down the starches into glucose units with the aid of enzymes, and those glucose molecules can circulate in the blood stream as an energy source. An interesting example of this enzyme-catalyzed breakdown process is if you chew on a piece of bread for a while, it will begin to taste sweet because the enzymes in saliva are already beginning to break down the starch into glucose, a sugar.

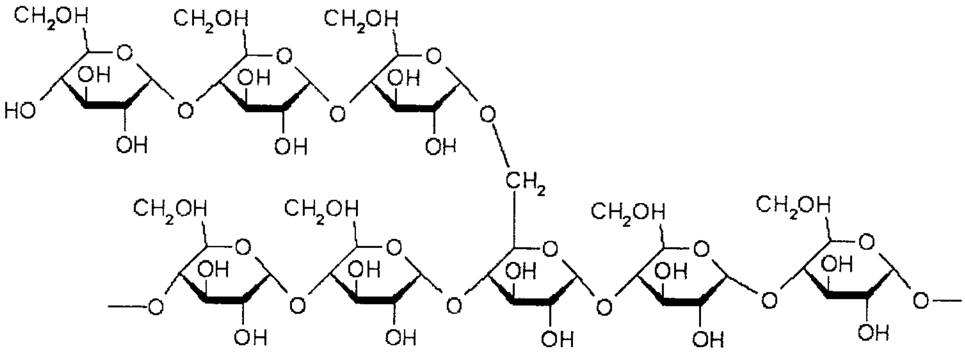
Inulin

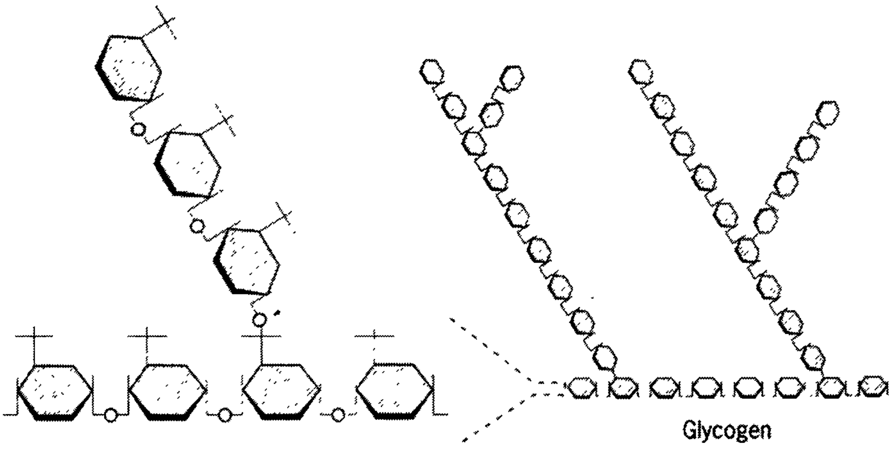
Inulin is a non-digestible fructosyl oligosaccharide found naturally in more than 36000 types of plants. It is a storage polysaccharide found in onion, garlic, chicory, artichoke, asparagus, banana, wheat and rye. It consists of mainly, if not exclusively, of β- 2→1 fructosyl-fructose links. A starting glucose moiety can be present, but is not necessary. Inulin is a soluble fibre that helps maintain normal bowel function,decreases constipation, lowers cholesrerol and triglycerides. It is used for fat replacement and fibre enrichment in processed foods.



Glycogen: animal starch. Just like amylopectin, but more highly branched (every 8-12 residues). This allows more free ends for more rapid breakdown-important in animals.

(1->4)-alpha-D-glycopyran with alpha 1->6 branching
Glycogen has more branching than amylopectin





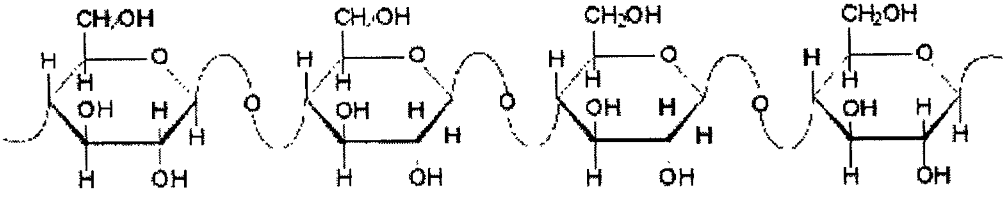
Structural Polysaccharides

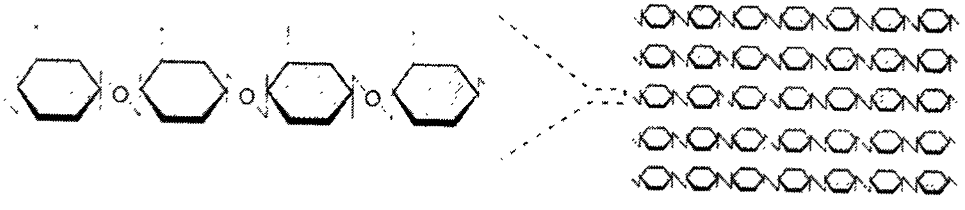
Cellulose:

Cellulose is the most abundant organic substance found in nature. It is a linear polymer of glucose, with 500 - 5000 glucose units linked together to give molecules of molecular weight 100,000 to 1,000,000. It is the principal constituent of cell walls in higher plants. It occurs in almost pure form (98%) in cotton fibres and to a lessor extent in flax (80%), jute (60-70%), wood (40-50%) and cereal straws (30-43%).

β-1,4 linkages, thus resistant to breakdown (including acid hydrolysis) as required for structure (do not participate in digestion). Multiple, extended strands come together as fibrils held together with H-bonds, laid down in cell wall in criss-cross pattern, glued together with polyalcohols known as lignin.

Cellulose is a form of carbohydrate in which some 1500 glucose rings chain together. It is the chief constituent of cell walls in living organisms. Wood is mostly cellulose, making cellulose the most abundant type of organic compound on the Earth.



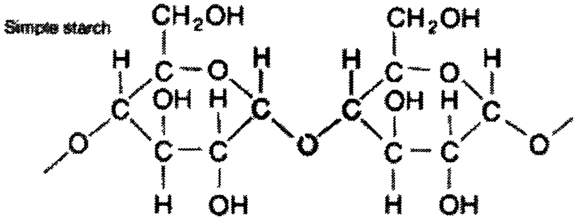


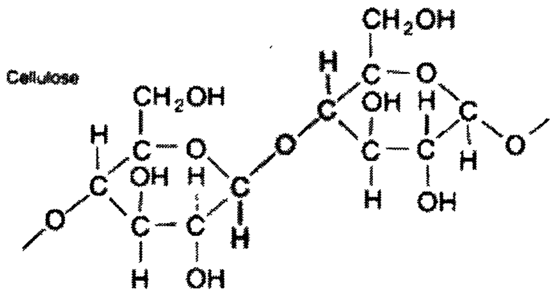
Cellulose

Cellulose molecules tend to be straight chains, and the fibers which result from collections of cellulose molecules have the strength to form the supporting structures of plants. Even though human digestion cannot break down cellulose for use as a food, animals such as cattle and termites rely on the energy content of cellulose. They have protozoa and bacteria with the necessary enzymes in their digestive systems. Cellulose in the human diet is needed for fibre.

The most stable conformation for the polymer is the chain turned 180° relative to the adjacent glucose residues yielding a straight extended chain. Celluose molecules within the plant cell walls are organized into biological units of structure known as microfibrils.

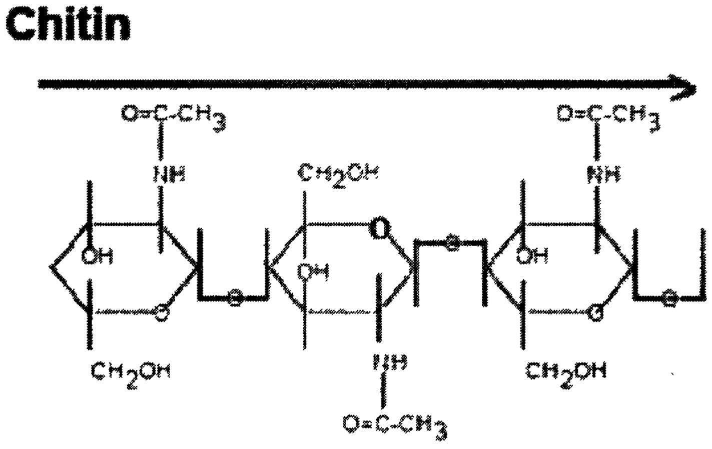
Comparison of Starch and Cellulose





Both starches and cellulose are carbohydrates which are classified as polysaccharides since they are composed of chains of glucose molecules. While they are similar, starches can be used as energy sources by the human body while cellulose cannot. Enzymes are important in the metabolism of foods, and these enzymes are very specific. They are somewhat like keys which will fit the geometry of the starch bonds, but not those of the cellulose bonds.

Chitin: Serves similar role to cellulose, but in animals (crustaceans and insects), fungi, and some algae. It is homopolymer of N-acetyl-D-glucosamine. Like cellulose, it has β-1,4 linkages, and is thus resistant to breakdown.



Chitin is a heteropolysaccharides, which is made up of **glycans** such as Hyaluronic acid, an alternating polysaccharide of D-glucuronic acid and N-acetyl-D-glucosamine; MW to 5,000,000 which serves as a lubricant in joints and is a component of the vitreous humor.

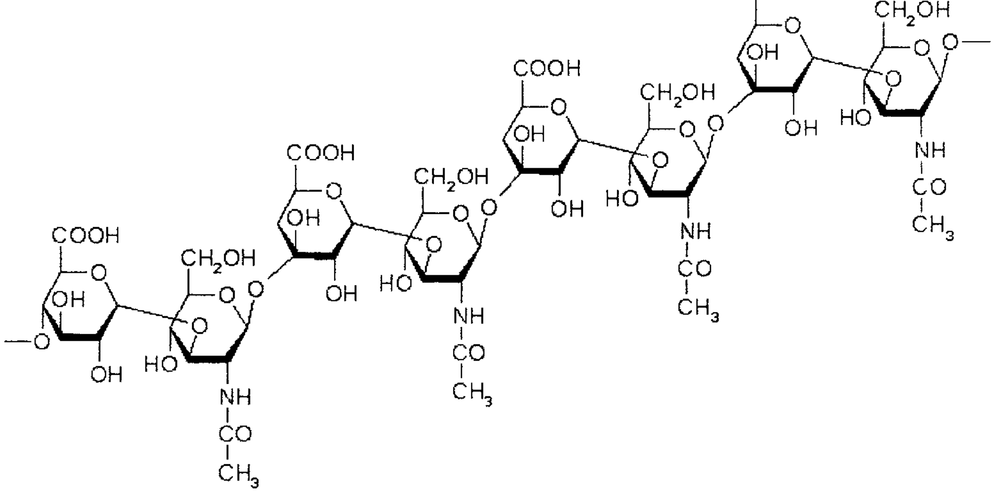
Also very important are the glycans conjugated to proteins and peptides to give proteoglycans.

Pectins and Hemicelluloses

Plants also contain more exotic polysaccharides in their cell walls, including pectins and hemicelluloses. Animals likewise have hyaluronan, and other glucosaminoglycans (GAG) in their extracellular matrices. GAGs and pectins are sugar/sugar-acid/sugar-amine polymers, and hemicellulose is a small polymer of glucose with variable amounts of rhamnose, galactose, xylose, mannose, fucose, and others. Their structures are somewhat complex.

Structural Unit

((1->4)-beta-N-acetyl-D-glucosamino)-beta-(1->3)-D-glucuronan



D-galacturonic acid residues form most of the molecules, in blocks of 'smooth' and 'hairy' regions. The molecule does not adopt a straight conformation in solution, but is extended and curved ('worm like') with a large amount of flexibility. The `hairy' regions of pectins are even more flexible and may have pendant arabinogalactans. The carboxylate groups tend to expand the structure of pectins as a result of their charge, unless they interact through divalent cationic bridging (their pKa of about 2.9 ensuring considerable negative charge under most circumstances). Methylation of these carboxylic acid groups forms their methyl esters, which take up a similar space but are much more hydrophobic and consequently have a different effect on the structuring of the surrounding water. The properties of pectins depend on the degree of esterification, which is normally about 70%. Low methoxyl-pectins (< 40% esterified) gel by calcium di-cation bridging between adjacent two-fold helical chains forming so-called 'egg-box' junction zone structures so long as a minimum of 14-20 residues can cooperate. It may well be that the two carboxylate groups have to cooperate together in prizing the bound water away from the calcium ions to form the salt links that make up these junction zones. The gelling ability of the di-cations is similar to that found with the alginates (Mg2+ << Ca2+, Sr2+ < Ba2+) with Na+ and K+ not gelling. If the methoxyl esterified content is greater than about 50%, calcium ions show some interaction but do not gel. The similarity to the behaviour of the alginates is that poly-α-(1,4)-D-galacturonic acid is almost the mirror image of poly-α-(1□4)-L-guluronic acid, the only difference being that the 3-hydroxyl group is axial in the latter. The controlled removal of methoxyl groups, converting high methoxyl pectins to low-methoxyl pectins, is possible using pectin methylesterases but the reverse process is not easily achieved.

High methoxyl-pectins (> 43% esterified) gel by the formation of hydrogen-bonding and hydrophobic interactions in the presence of acids and sugars.

Function of Pectin

Pectins are mainly used as gelling agents, but can also act as thickener, water binder and stabilizer.

Low methoxyl pectins (< 50% esterified) form thermoreversible gels in the presence of calcium ions and at low pH (3 - 4.5) whereas high methoxyl pectins rapidly form thermally irreversible gels in the presence of sufficient (e.g., 65% by weight) sugars such as sucrose and at low pH (< 3.5); the lower the methoxyl content, the slower the set.

The degree of esterification can be (incompletely) reduced using commercial pectin methylesterase, leading to a higher viscosity and firmer gelling in the presence of Ca2+ ions. Highly acetylated pectin from sugar beet is reported to have considerable emulsification ability due to its more hydrophobic nature, but this may be due to protein impurities.

As with other viscous polyanions such as carrageenan, pectin may be protective towards milk casein colloids, enhancing the properties (foam stability, solubility, gelation and emulsification) of whey proteins whilst utilizing them as a source of calcium.

Pectin Substances

Pectin substances are natural components of plants and their fruits. They occur in plants in connection with cellulose and such substances are called protopectin. Protopectin is the binder of cell walls. Especially large amounts of pectin substances are present in fruit such as: currant, gooseberry, citrus fruits and apples. Pectin is a preparation obtained in industrial conditions, containing pectin substances isolated from plant material and soluble in water. Those preparations are used as food and medicine additives and they have the ability to make gels in proper conditions. Raw material for our pectin is dried apple pomace, containing 8-12 % pectin substances, and dried lemon peel, containing 18-25 % pectin substances, from where they are extracted by diluted acid solution and subsequently precipitated by alcohol, purified, dried and crumbled. Being the substance of plant origin, it is the best gelling agent for jams and fruit jellies production. Being the naturally compound of fruit, it makes products manufactured with its addition retain fully organoleptic characteristics.

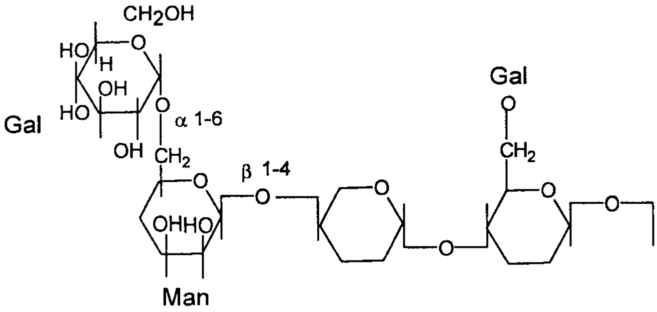
Gums

- Gums are hydrocolloids High affinity for H_2O.
- More complex in structure than starch or pectin.
- Primary building block is galactose (1000 unit).
- They are plant extractives, not digested but are soluble fibres -Low calorie foods.

Sources are -

Guar gum - Cyamopsis tetragonolobus
Taragacanth - Astragalaus gummifer
Locust bean gum (Carob) - Ceratonia siliqua
Gum Karaya - Sterulia urens
Xanthan gum (bacterial)
Gum Arabic - Acacia Senegal

Guar gum
Galactomannan
Mannose β 1 → 4
Galactose α 1 → 6
(every other mannose)



Xanthan gum
Glucose 2.8 moles
Mannose 3.0 moles
Glucuronic 2.0 moles
All β 1→4 linkage
Backbone similar to cellulose
Trisaccharide side chain at 3 position alternate glucose

Locust bean gum
Galactomannan
Galactose for every 4th mannose

Fenugreek gum (Trigonella foenum -graecum)
Monnose and galactose 1:1
Prosopsis juliflora gum man: Gal 5:4

Typical Comparison of Storage and Structural Polysachharides

Storage polysaccharides	Structural polysaccharides
Occurs in the storage organs such as seeds and tubers.	Important constituents of plant cell wall.
Include both homo and heteropolysaccharides	Include both homo and heteropolysaccharides.
Starch and inulin are homopolysaccharides made from glucose and fructose, respectively.	Cellulose is a homopolysaccharide made up of glucose.
Arabinogalactan is an example for storage heteropolysaccharide.	Hemicelluloses are heterpolysaccharides containing pentoses, hexoses and monosaccharide derivatives.
The configuration of major linkages are α-type.	The configuration of major linkages are β-type.

Chemical Properties of Carbohydrates

- Reactions of monosaccharides are due to the presence of hydroxyl (-OH) and the potentially free aldehyde (-CHO) or keto (>C=O) groups.
- Sugars in weak alkaline solutions undergo isomerization to form 1,2-enediol followed by the formation of a mixture of sugars.
- Under strong alkaline conditions sugar undergo caramelization reactions.
- Sugars are classified as either reducing or non-reducing depending upon the presence of potentially free aldehyde or keto groups. The reducing property is mainly due to the ability of these sugars to reduce metal ions such as copper or silver to form insoluble cuprous oxide, under alkaline condition. The aldehyde group of aldoses is oxidized to carboxylic acid. This reducing property is the basis for qualitative (Fehling's, Benedict's, Barfoed's and Nylander's tests) and quantitative reactions. All monosaccharides are reducing. In the case of oligosaccharides, if the molecule possesses a free aldehyde or ketone group it belongs to reducing sugar (maltose and lactose). If the reducing groups are involved in the formation of glycosodic linkage, the sugar belongs to the non- reducing group (trehalose, sucrose, raffinose and stachyose).
- When reducing sugars are heated with phenylhydrazine at pH 4.7 a yellow precipitate is obtained. The precipitated compound is called as osazone. One molecule of reducing sugar reacts with three molecules of phenylhydrazine.
- D-mannose and D-fructose form same type of osazone as that of D-glucose since the configuration of C-3, C-4, C-5 and C-6 is same for all the

three sugars. The osazone of D-galactose is different. Different sugars form osazone at different rates. For example, D-fructose forms osazone more readily than D-glucose. The osazones are crystalline solids with characteristic shapes, decomposition points and specific optical rotations. The time of formation and crystalline shape of osazone is utilized for identification of sugars. If methyl phenylhydrazine is used instead of phenylhydrazine in the preparation of osazone, only ketoses react. This reaction serves to distinguish between aldose and ketose sugars.

- The hydroxyl group formed as a result of hemiacetal formation in monosaccharides react with methanol and HCl to form methyl α- and β-glycosides. The derivaties of each sugar are named according to the name of the sugar, that is, the derivaties of glucose as glucosides, of galactose as galactosides and of arabinose as arabinosides etc. Glycosides are acid-labile but are relatively stable at alkaline pH. Since the formation of glycosides convert the aldehydic group to an acetal group, the glucosides are not a reducing sugars.
- Glycosides are also formed with a non-sugar component, the aglycone. The sugars which are connected to the non-sugar moiety, are pentoses, hexoses, branched sugars or deoxy or dideoxy sugars. The chain length varies from one to five monosaccharide sugar residues per glycosides. Apart from O-glycosides, three other classes of glycosides are found in higher plants namely S-glycosides, N-glycosides and C-glycosides.
- Monosaccharides are generally stable to hot dilute mineral acids though ketoses are appreciably decomposed by prolonged action.
- Heating a solution of hexoses in a strong non-oxidising acidic conditions, hydroxy methyl furfural is formed. The hydroxymethyl furfural from hexose is usually oxidized further to other products When phenolic compounds such as resorcinol, α-naphthol or anthrone are added, mixture of coloured compounds are formed .
- The molisch test used for detecting carbohydrate in solution is based on following principle. When conc. H_2SO_4 is added slowly to a carbohydrate solution containing α-naphthol, a pink colour is produced at the juncture. The heat generated during the reaction hydrolyse and dehydrate it to produce furfural or hydroxymethyl furfural which then react with α-naphthol to produce the pink colour.
- When sugars are treated with appropriate acid anhydride or acid chloride under proper conditions, the hydroxyl groups get esterified and form sugar esters.

QUIZ

Identify the letter of the choice that best completes the statement or answers the question.

1. Two sugars that differ in configuration only at one chiral center are called
   A. enantiomers B. diastereomers
   C. anomers D. epimers

Answer: D

2. Cellulose is
   A. a linear polymer of glucose units linked by beta(1-4) glycosidic bonds
   B. a linear polymer of glucose units linked by alpha(1-4) glycosidic bonds
   C. a branched polymer of glucose units linked by both alpha(1-4) and beta(1-4) linkages
   D. a linear polymer of fructose units linked by alpha(1-6) linkages

Answer: A

3. Differences between amylose and amylopectin include
   A. Amylose contains only glucose residues, while amylopectin contains both glucose and fructose.
   B. Amylopectin is a branched molecule, while amylose is linear.
   C. Amylose has both alpha(1-4) and alpha(1-6) glycosidic linkages, while amylopectin has only alpha(1-4) linkages.
   D. Amylose has alpha(1-4) glycosidic linkages, while amylopectin has beta(1-4) linkages.

Answer: B

4. The repeating units in chitin are
   A. mannose units B. glucose units
   C. L-glucuronate units D. N-acetylglucosamine units

Answer: D

5. D-Ribose is:
   A. a hexose B. a ketose
   C. a pentose D. a deoxy sugar

Answer: C

6. Two sugars that differ in configuration at only one chiral center are described as
   A. epimers B. hemiketals
   C. diastereomers D. enantiomers

Answer: A

7. In a sugar, the end of the molecule containing the free anomeric carbon is called

A. the epimeric center B. the glycosidic bond
C. an acetal D. the reducing end

Answer: D

8. Sucrose is a non-reducing sugar because
A. It contains a free anomeric carbon and therefore cannot mutarotate.
B. It has a beta(-6) glycosidic linkage.
C. It gives rise to fructose and glucose upon hydrolysis.
D. It does not contain a free anomeric carbon atom.

Answer: D

9. Beta-D-glucopyranose behaves in its chemical properties like
A. an acetal B. a ketal
C. a hemiacetal D. an amine

Answer: C

10. Amylopectin, when hydrolyzed in the presence of a-amylase, yields maltose and limit dextrins. However, amylose, under the same conditions, almost quantitatively produces maltose. This difference in behavior is best explained by:
A. the greater solubility of amylose in water
B. differences in the molecular weights of amylose and amylopectin
C. the presence of more glucose units in the amylose molecule
D. the presence of substantial numbers of a-1-6 linkages in amylopectin and none in amylose

Answer: D

14. Which of the following is NOT a major storage molecule for animal tissues?
A. protein B. glycogen
C. triaclyglycerols D. cellulose

Answer: D

15. The molecular formula for glucose is $C_6H_{12}O_6$. What would be the molecular formula for a polymer made by linking ten glucose molecules together by dehydration synthesis?
A. $C_{60}H_{120}O_{60}$ B. $(C_6H_{12}O_6)_{10}$.
C. $C_{60}H_{102}O_{51}$ D. $C_{60}H_{100}O_{50}$.

Answer: C

16. Monosaccharides, such as ribose, fructose, glucose, and mannose differ significantly in
A. their sweetness B. the positions of their carbonyl groups.
C. their diastereomeric configurations. D. All

Answer: D

17. Boat and chair conformations are found
A. in pyranose sugars.

B. in furanose sugars.
C. in any sugar without axial -OH groups.
D. in any sugar without equatorial -OH groups.

Answer: A

18. Which of the following is an example of a storage polysaccharide made by animals?
A. Cellulose. B. Glycogen.
C. Collagen. D. Starch.

Answer: B

19. Cellulose, a b(1->4)-linked glucose polysaccharide, differs from starch in that starch is
A. A b(1->6)-linked manose polysaccharide
B. A b(1->6)-linked glucose polysaccharide.
C. An a(1->6)-linked glucose polysaccharide.
D. An a(1->4)-linked glucose polysaccharide.

Answer: D

20. The glycosidic bond
A. joins glucose and fructose to form sucrose.
B. in maltose is not hydrolyzed in "lactose intolerant" humans.
C. in lactose is hydrolyzed by human infants to make two galactose.
D. None

Answer: A

21. Cellulose fibers resemble ___ in proteins; whereas a-amylose is similar to ___.
A. a-helices; b-sheets.
B. b-sheets; a-helices.
C. b-sheets; the hydrophobic core.
D. a-helices; b-turns.

Answer: B

22. The N-linked glycoproteins of eukaryotes usually have a N-acetyl glucosamine (NAG) attached to
A. a surface Asn residue.
B. a surface Gln residue.
C. a buried Asn residue.
D. the amino terminal residue.

Answer: A

23. The O-linked glycoproteins of eukaryotes usually have their sugar chains attached to
A. buried carbonyls in the protein backbone.
B. surface carbonyls in the protein backbone.
C. the OH of Ser or Thr residues.

D. the carboxyl terminal residue.

Answer: C

24. Starch is a polymer made from the following monomer
A. β-glucose B. α-glucose
C. α-fructose D. α-galactose

Answer: B

25. The products of hydrolysis of lactose are:
A. α-glucose and α-fructose B. α-glucose and α-galactose
C. α-fructose and α-galactose D. α-galactose and α-ribose

Answer: B

26. The type of bond that forms when a disaccharide is formed from two monosaccharides is called:
A. A peptide bond B. A carbohydrate bond
C. An ester bond D. A glycosidic bond

Answer: D

27. The type of reaction that occurs when a disaccharide is formed from two monosaccharides is
A. Addition B. Hydrolysis
C. Condensation D. Reduction

Answer: C

28. Which is the most important carbohydrate fuel in human cells?
A. Ribose B. Glucose
C. Galactose D. Fructose

Answer: B

29. Which two monosaccharides combine to form sucrose?
A. α-galactose and α-fructose B. α-fructose and α-ribose
B. α-glucose and β-glucose D. α-glucose and α-fructose

Answer: D

30. Aldoses are reducing sugars because in their non-cyclic form they contain:
A. A ketone group B. An ester group
C. An hydroxyl group D. An aldehyde group

Answer: D

31. Saccharides contain the following combination of elements:
A. carbon, hydrogen and phosphorus
B. carbon, nitrogen and hydrogen
C. carbon, oxygen and hydrogen
D. carbon and hydrogen

Answer: C

Complete each sentence or statement.

1. Sugars that differ only by the configuration at one C atom are ________.

Answer: epimers

2. A/An ________ is any six-carbon sugar.

Answer: hexose

3. A/An ________ consists of two opposing metabolic reactions that function together to provide a control point for regulating metabolic flux.

Answer: futile cycle or substrate cycle

4. A/An ________ is a sugar with the structure of an aldehyde.

Answer: aldose

5. A/An ________ is the linkage between the anomeric carbon of a saccharide and an alcohol or amine.

Answer: glycosidic bond

6. Pentose is a ________ carbon sugar.

Answer: five

7. The ______ and ______ stereoisomers of sugars refer to the configuration of the asymmetric carbon atom from the aldehyde or ketone group.

Answer : D, L

8. A ______________ is formed when two monosaccharides become joined by a glycosidic bond.

Answer : disaccharide

9. The _________ groups of sugars can be replaced by other groups to form a wide range of biologically important molecules including phosphorylated sugars, amino sugars and nucleotides.

Answer : hydroxyl

10. Lactose is a disaccharide formed between C-1 of ____________ and C-4 of __________.

Answer : galactose, glucose

11. Long chains of monosaccharide joined together are collectively called _______________.

Answer : polysaccharide

12. __________ is a branched chain polysaccharide containing glucose residues linked by α 1-4 bonds with α 1-6 branch point.

Answer : Glycogen

13. __________ is a mixture of unbranched amylase and branched amylopectin.

Answer : Starch

14. _________ consists of glucose residue linked mainly by α 1-6 bonds.

Answer : Dextran

15. __________ is a straight chain polymer of glucose units linked by β 1-4 bonds.

Answer : Cellulose

16. Short chains of monosaccharides linked by glycosidic bonds are called ____________.

Answer : oligosaccharides

17. Commercially ___________ is manufactured by the hydrogenation of glucose.

Answer : sorbitol

18. __________ occurs in many terrestrial and marine plants.

Answer : Mannitol

19. ___________ is an excellent moisture conditioner and is used in pharmaceutical preparations like elixirs and syrups.

Answer : Sorbitol

20. ________ acids are formed when aldoses are oxidized with nitric acid.

Answer : Aldaric or Saccharic

21. Oxidation of an aldose with bromine water at neutral pH converts the aldehyde group to a _________ group and resulted to form ___________.

Answer : carboxyl, aldonic acid

22. _________ is commercially prepared from mannose and fructose.

Answer : Mannitol

23. _________ sugar is found in milk.

Answer : Lactose

24. _________ sugar is stored as a reserve food supply in insect's hemolymph.

Answer : Trehalose

25. ________ is a non-digestible fructosyl oligosaccharide found naturally in more than 36,000 types of plants.

Answer : Inulin

Indicate whether the sentence or statement is true (T) or false (F).

1. The body's favorite energy supply is from protein.

Answer: F

2. Carbohydrates can be sugars, starches or fiber.

Answer: T

3. Whole-grain breads, cereals and pastas are good sources of carbohydrate.

Answer: T

4. Sugars that have three or more sugar molecules are called monosaccharides.

Answer: F

5. The number of carbohydrate grams in a food can be found on the Nutrition Facts Label.

Answer: T

6. Sucrose is a reducing sugar.

Answer: F

7. Sugar found in human blood is glucose

Answer: T

8. Molisch reaction is a general test for carbohydrates

Answer: T

9. Iodine test for polysaccharides is a chemical reaction

Answer: F

10. The changes in specific rotation of an optically active solution without any change in other properties is known as mutarotation.

Answer: T

11. Non carbohydrate protion of a glycoside is called aglycone.

Answer: T

12. Dextrose is the partially degraded breakdown products of starch.

Answer: F

13. Sucrose is called an invert sugar.

Answer: T

14. Carbohydrates that differ in their configuration around a specific carbon atom than the carbonyl carbon atom are called epimers.

Answer: T

15. Carbohydrates that differ only in their configuration around the carbonyl carbon atom are called anomers.

Answer: T

"This Page is Intentionally Left Blank"

Chapter **3**

Lipids and Membranes

The word lipids is derived from the Greek word 'lipos' meaning fat. Lipids are chemically heterogenous group of compounds that are insoluble in water but soluble in non-polar solvents such as hexane, chloroform. Lipids occur in plants and animals as storage and structural components. Structural lipids present in animals and plants are in the form of meat and vegetables, respectively. Storage fats occur in milk and adipose tissue of farm animals and in seed oils. Fats supply over twice as much energy per unit weight as proteins or carbohydrates. Lipids are anhydrous due to non-polar nature and represent more energy than carbohydrates which are heavily hydrated due to polar nature. The presence of lipids in diet contributes considerably to palatability. A fat- free diet would be unpleasant to eat. Lipids contribute palatability in two ways. They induce olfactory responses, namely, taste in the mouth and aroma through nose. Secondly, they contribute to the texture of food and is responsible for the mouth-feel. Lipids also supply the essential fatty acids which are not synthesised in human beings but are essential for growth. Lipids are essential for the effective absorption of fat-soluble vitamins A, D, E and K from intestine. Many enzymes require lipid molecules for maximal activity. Examples are microsomal enzyme, glucose 6-phosphatase and mitochondrial enzyme, β -hydroxybutyrate dehydrogenase. Adrenal corticosteroids, sex hormones and vitamin D_3 (Cholecalciferol) are synthesized from lipid derivative (cholesterol). Much of the lipid of mammals is located subcutaneously and acts as insulation against excessive heat loss to the environment.

Classification of Lipids

Simple Lipids

- Esters of fatty acids with glycerol and monohydric alcohols.
- Depending upon the constituent alcohols they are further subdivided into fats or oils and waxes.
- Fats, also termed as triacylglycerols are esters of fatty acids with glycerol e.g., Plants–vegetable oils; Animals–ghee and butter.
- Waxes are esters of fatty acids and alcohols other than glycerol e.g., Plant wax-carnâuba wax; Insect wax–beeswax; Animal wax -lanolin.

Compound Lipids

- Esters containing chemical groups in addition to alcohol and fatty acids.
- Depending upon the chemical groups they are further subdivided into phospholipids, glycolipids, sulpholipids and lipoproteins.
- Phospholipids contain phosphate group. Phopholipids are further grouped as glycerophospholipids, if the constituting alcohol is glycerol (e.g., Lecithin) or as sphingophospholipids, if the alcohol is sphingosine (e.g., Sphingomyelin).
- Glycolipids contain hexose units preferably galactose alongwith fatty acids and alcohol e.g., cerebrosides.
- Plant sulpholipids contain sulfated hexose with fatty acids and alcohol.
- Lipoproteins contain protein subunits along with lipids. Depending upon density and lipid compound they are further classified as VLDL. LDL and HDL.

Derived Lipids

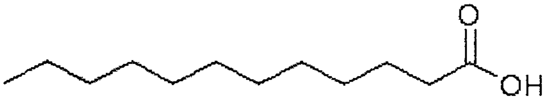
- Substances derived from simple and compound lipids by hydrolysis of alcohols, fatty acids, aldehydes, ketones, sterols and hydrocarbons.

Lipids are water-insoluble that are either hydrophobic (nonpolar) or amphipathic (polar and nonpolar regions). Lipids are in many ways the most diverse of the biological macromolecules, since they are something of a rag-tag bunch of leftovers. Lipids are pretty much everything in the cell that isn't very water soluble, and chemically they don't have a great deal in common with one another. The best known lipids are probably the fatty acids, so that is where we shall start.

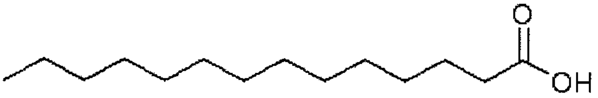
The Fatty Acids

The fatty acids are long chain carboxylic acids synthesised by the condensation and reduction of acetyl coenzyme-A units by fatty acid synthase. The more important ones have nonsystematic names in wide use. Stearic and palmitic acids are ***saturated*** (no double bonds), oleic acid is ***monounsaturated,*** and linoleic and linolenic are ***polyunsaturated*** (Table 3.1). All these common fatty acids are *cis* (E) fatty acids. Because of the links in the chain caused by the double bonds, the unsaturated fatty acids tend to be liquids at room temperature (they are less easy to pack together to form a solid). Bacteria and plants (which cannot thermoregulate) will use more unsaturated acids in their cell membranes when they are exposed to cold: this helps to maintain membrane fluidity.

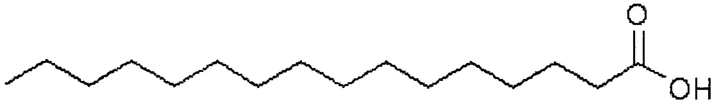
Molecular structure **Name of Fatty acid**



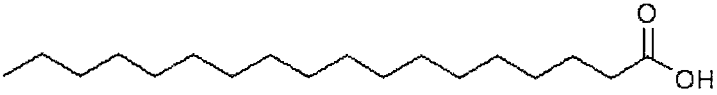
Lauric acid: saturated C_{12}



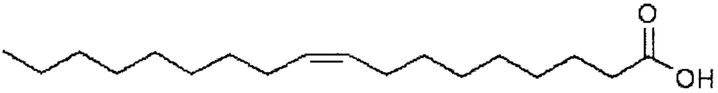
Myristic acid: saturated C_{14}



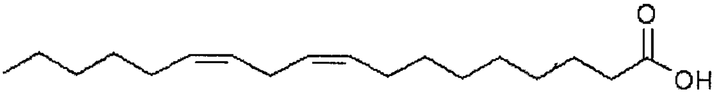
Palmitic acid: saturated C_{16}



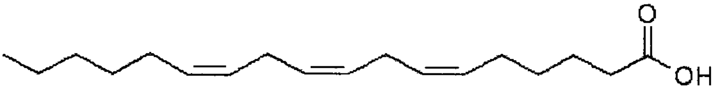
Stearic acid : saturated C_{18}



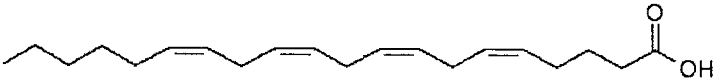
Oleic acid : monounsaturated C_{18}



Linoleic acid : diunsaturated C_{18}



γ -Linolenic acid : triunsaturated C_{18}



Arachidonic acid : tetraunsaturated C_{20}

The nomenclature of these acids is rather complicated. There are at least five systems in use. Here are some of the above in the different systems. The delta system numbers the double bonds from the carboxyl group (the Δ carbon), whereas the omega system indicates where the first double bond is counting from the other end of the molecule (the ω carbon).

Table 3.1 : Details of important Saturated and Unsaturated Fatty Acids.

Fatty Acid	Systematic	Colon	Delta	Omega
Stearic acid	Octadecanoic acid	18:0	Octadecanoic acid	-
Palmitic acid	Hexadecanoic acid	16:0	Hexadecanoic acid	-
Oleic acid	E-Octadec-9-enoic acid	18:1n9	cis-Δ^{9}-octadecenoic acid	ω9
Linoleic acid	9E, 12E-Octadeca-9, 12-dienoic acid	18:2n9	cis, cis-$\Delta^{9, 12}$-octadecadienoic acid	ω6
Linolenic acid	6E, 9E, 12E-Octadeca-6, 9, 12-trienoic acid	18:3n6	cis, cis, cis -$\Delta^{6,9,12}$-octadecatrienoic acid	ω3

However, the most important use for lipids in the cell is in the formation of membranes. membranes contain amphipathic molecules, *i.e.,* ones with a hydrophobic end *and* a hydrophilic end.

Some of these lipids contain two fatty acids bound to a glycerol molecule. These are glycerolipids. The third position is taken up by a polar head group containing phosphate and another compound. This other compound can be choline (as below) forming *phosphatidyl choline,* or serine (*phosphatidyl serine*), glycerol, inositol, ethanolamine, hydrogen (*phosphatidic acid*) or even an entire other diacylglycerol unit (forming a *cardiolipin*).

Essential Fatty Acids

Human body is unable to synthesise all fatty acids found in the body. Those fatty acids that are not synthesized in the body but required for normal body growth and maintenance are called as essential fatty acids. These fatty acids are to be supplied through diet. Linoleic and linolenic acids are essential fatty acids. The longer chain fatty acids can be synthesized by the body from dietary linoleic and α-linolenic acids. Arachidonic acid is essential but it can be synthesised by the body from linolenic acid. It is also present in the meat. Linoleic acid is grouped under n-6 family because the 6th carbon from methyl end possesses the double bond.

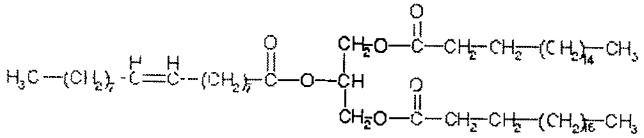
Simple Lipids

Lipids containing only fatty acids and glycerol or long chain alcohols (monohydric) are called as simple lipids which include fats, oils and waxes.

Fats and Oils

Triacylglycerols are the simplest lipids constructed from fatty acids and glycerol. They are also referred as triglycerides, fats or neutral fats. Triacylglycerols are composed of three fatty acids esterified to the three hydroxyl groups of glycerol. Triacylglycerol that are solid at room temperature are called as fats, while the liquid triacylglycerols are called as oils. Fats are usually rich in saturated fatty acids and the unsaturated fatty acids predominate in oils. Most oil-producing plants store their lipids in the form of triacylglycerols.

Three fatty acids esterified to glycerol known as triglycerides


CH2—O—C(=O)—CH2—CH2—(CH2)14—CH3
H3C—(CH2)7—CH=CH—(CH2)7—C(=O)—O—CH
CH2—O—C(=O)—CH2—CH2—(CH2)16—CH3

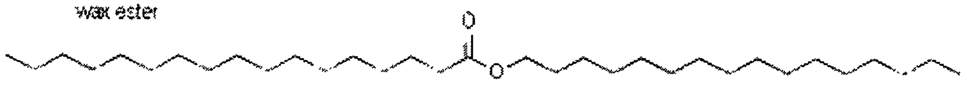
A triacylglycerol

Structural or Hidden Fats in Plants

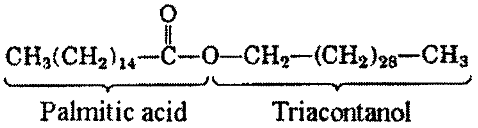
The leaves of higher plants contain upto 7% of their dry weight as fats; some of which are present as surface lipids, the others as components of leaf cells, especially in the chloroplast membrane. The fatty acid composition of plant membrane lipids is very simple. Six fatty acids- palmitic, palmitoleic, stearic, oleic, linoleic and γ-linolenic generally account for over 90% of the total fatty acids.

Waxes

Waxes are esters of long-chain saturated and unsaturated fatty acids with long chain alcohol (Table 3.2). The carbon number of fatty acids vary from 14 to 34 and that alcohol from 16 to 30.



For example, beeswax is an ester of palmitic acid with a 30 carbon alcohol, triacontanol.



$$\underbrace{CH_3(CH_2)_{14}-\overset{\overset{\displaystyle O}{\|}}{C}}_{\text{Palmitic acid}}-\underbrace{O-CH_2-(CH_2)_{28}-CH_3}_{\text{Triacontanol}}$$

More commonly, waxes are esters of an alcohol other than glycerol (long chain alcohol, sterol, hydroxycarotenoids, vitamin A) and a long chain acid (wax esters). Wax esters are saponified by hot alkaline solutions and give a fatty acid and an alcohol. They are soluble in aromatic solvents, chloroform, ethers, esters and ketones.

Table 3.2 : Wax Components and Its General Structure

Compound	General structure
n-Alkanes	$H_3C[CH_2]_nCH_3$
Ketones	R^1COR^2
Secondary alcohols	$R^1CH\{OH)R^2$
β-Diketones	$R^1COCH_2COR^2$
Monoesters	R^1COOR^2
Primary alcohols	RCH_2OH
Aldehydes	$RCHO$
Alkanoic acids	$RCOOH$
Dicarboxylic acids	$HOOC[CH_2]_nCOOH$
ω-Hydroxy acids	$HOCH_2[CH_2]_nCOOH$

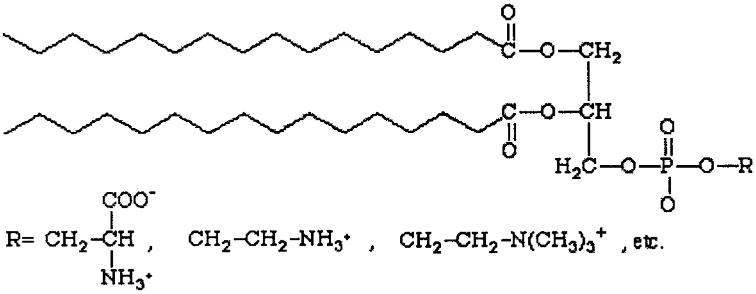
Waxes are the chief storage form of metabolic fuel in marine phytoplanktons. Biological waxes have find a variety of applications in the pharmaceutical, cosmetic and other industries. Waxes are not easily hydrolysed like fats or digested by lipases.

Compound Lipids

Compound lipids contain certain chemical groups in addition to alcohol and fatty acids. These group of lipids include glycerophospholipids, sphingo phospholipids, glycolipids, sulpholipids and lipoproteins.

Glycerophospholipids

The important structural lipid in biological membrane is glycero phospholipid which contains glycerol, fatty acids, phosphoric acid and a nitrogenous base. Two fatty acids and a phosphate esterified to glycerol

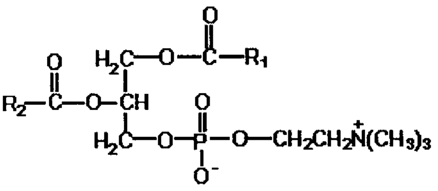


$R= CH_2\text{-}CH(COO^-)(NH_3^+)$, $CH_2\text{-}CH_2\text{-}NH_3^+$, $CH_2\text{-}CH_2\text{-}N(CH_3)_3^+$, etc.

Without alcoholic residue (R), it is called as phosphatidic acid. Depending on the alcoholic residue attached to phosphatidic acid, they are named as :-

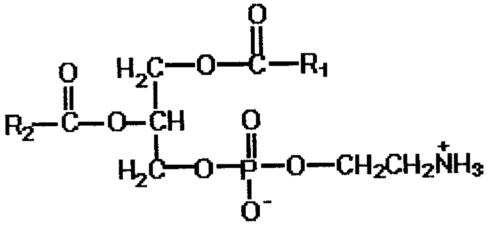
- Phosphatidyl choline (lecithin)
- Phosphatidyl ethanolamine (cephalin)
- Phosphatidyl serine
- Phosphatidyl inositol
- Phosphatidyl glycerol (which include monophosphatidyl glycerol and diphosphatidyl glycerol or cardiolipin).

Phosphatidyl choline (lecithin)



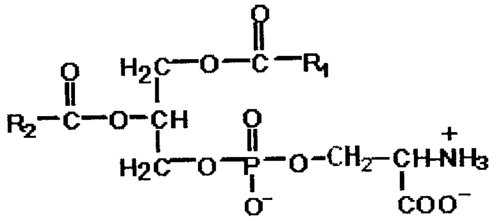
Lecithins are widely distributed in the membranes of cells having both metabolic and structural functions. Dipalmityl lecithin is a very effective surface active agent preventing adherence due to surface tension of the inner surfaces of the lungs. Most phospholipids have a saturated fatty acid in the C1 position but an unsaturated fatty acid in the C2 position.

Phosphatidyl ethanolamine (cephalin)



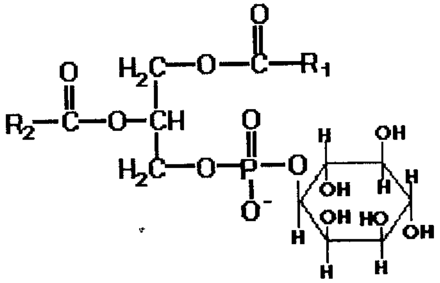
The cephalin differs from lecithin only in the nitrogenous group where ethanolamine is present instead of choline.

Phosphatidyl serine



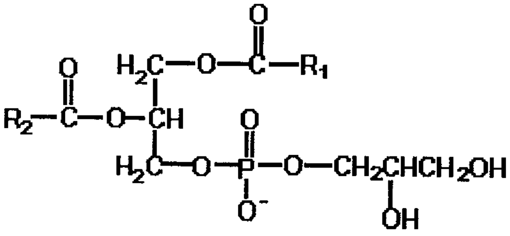
The hydroxyl group of the amino acid L-serine is esterified to the phosphatidic acid.

Phosphatidyl inositol

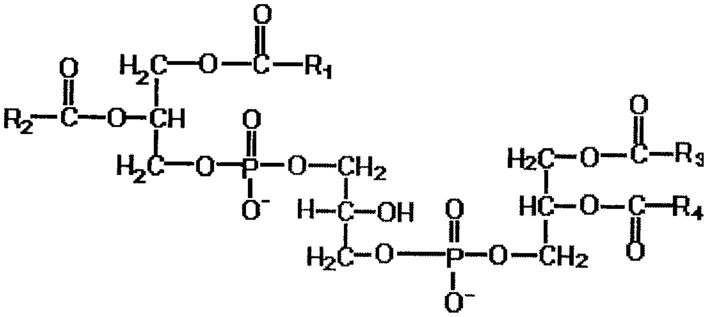


Phosphatidyl inositol is an important constituent of cell membrane phospholipids; upon stimulation by a suitable animal hormone it is cleaved into diacylglycerol and inositol phosphate, both of which act as internal signals or second messengers.

Phosphatidyl glycerol



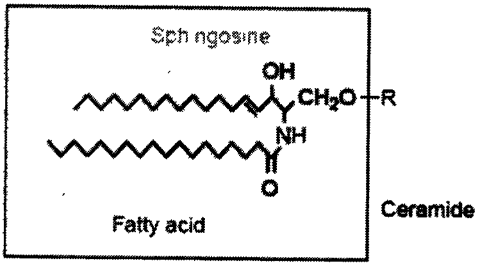
Cardiolipin is a phospholipid that is found in membranes of mitochondria. It is formed from phosphatidylglycerol.



Sphingophospholipids

The phosphate and fatty acids are attached to the alcohol sphingosine instead of glycerol in sphingophospholipids. The fatty acids are attached through an amide linkage rather than the ester linkage. The base present is normally choline. C-1, C-2 and C-3 of the sphingosine or phytosphingosine bear functional groups, -OH, -NH2 and -OH respectively, which are structurally homologous with the three hydroxyl groups of glycerol.

All sphingolipids contain a sphingoid long-chain base (*e.g.*, sphingosine) that is linked to a fatty acid molecule through an amide bond, thereby forming the ceramide unit. Addition of phosphocholine or carbohydrates to ceramide leads to sphingomyelin or glycosphingolipids, respectively." (Fig. 3.1)


Sphingosine
OH
CH₂O
R
NH
O
Fatty acid
Ceramide

Substituent (R)	Sphingolipid
H	Ceramide
Phosphocholine	Sphingomyelin
Sugar(s)	Glycosphingolipid

Fig. 3.1 . General sphingolipid Structure

Glycolipids and Sulpholipids

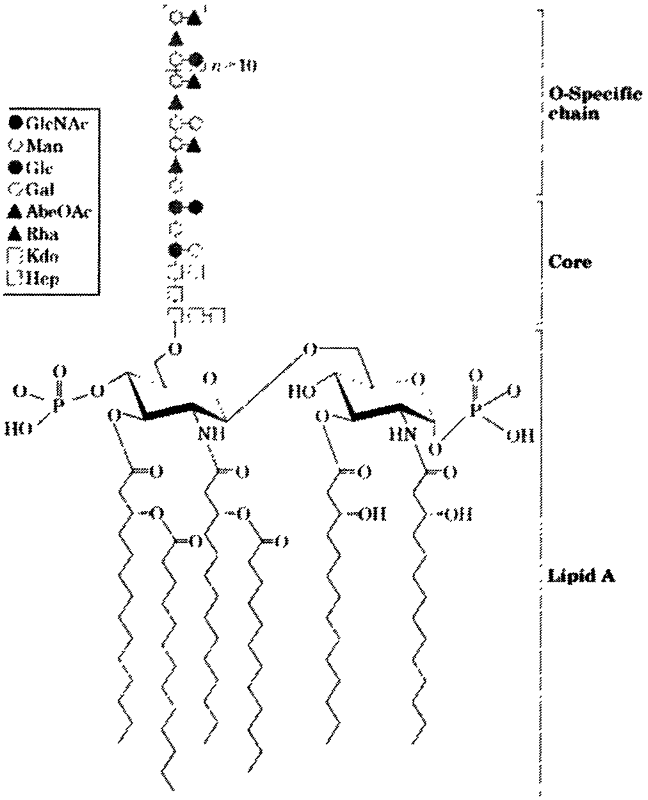
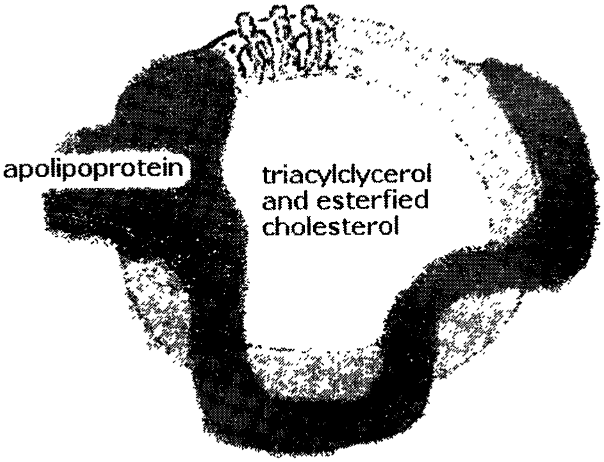

● GlcNAc
○ Man
● Glc
○ Gal
▲ AbeOAc
▲ Rha
□ Kdo
□ Hep
n ~ 10
O-Specific chain
Core
Lipid A
O
P
HO
NH
HN
OH

Fig. 3.2 : General Structure of Glycolipids

Glycolipids are structurally characterised by the presence of one or more monosaccharide residues and the absence of a phosphate (Table 3.2). They are O-glycoside of either sphingosine or glycerol derivative. The monosaccharides commonly attached are D-glucose, D-galactose or N-acetyl D-galactosamine. Monogalactosyl diglycerides and digalactosyl diglycerides have been shown to be present in a wide variety of higher plant tissues.

Lipoprotein

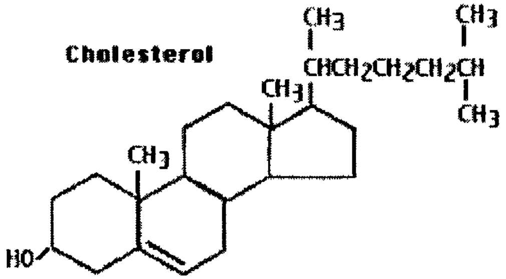


Protein molecules associated with triacylglycerol, cholesterol or phospholipids are called lipoproteins. Triacylglycerols derived from intestinal absorption or from the liver are not transported in the free form in circulating blood plasma, but move as chylomicrons, as very low density lipoproteins (VLDL) or as free fatty acids (FFA) - albumin complexes. Besides, two more physiologically important groups of lipoproteins are low density lipoprotein (LDL) and high density lipoprotein (HDL).

The major lipid components of chylomicrons and VLDL are triacylglycerol, whereas the predominant lipids in LDL and HDL are cholesterol and phospholipids respectively. The protein part of lipoprotein is known as apoprotein. Lipoproteins occur in milk, egg-yolk and also as components of cell membranes.

Sterols

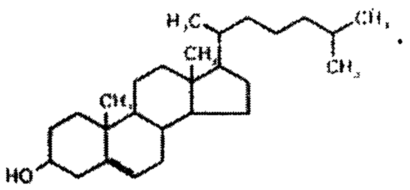
The characteristic structure of sterol is their steroid nucleus consisting of four fused rings, three with six carbons (Phenanthrene) and one with five carbons (cyclopentane). This parent structure is known as perhydro cyclopentano phenanthrene. **Cholesterol** is the most abundant sterol in animals. Cholesterol is a major component of animal plasma membranes and occurs in lesser amounts in the membranes of their subcellular organelles.



Cholesterol

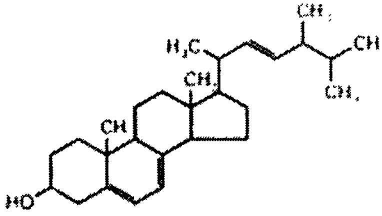
CH3
CH3
CHCH2CH2CH2CH
CH3
CH3
CH3
HO

Different Types of Cholesterol



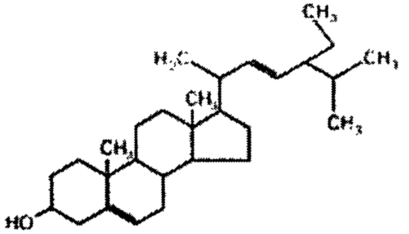
H3C
CH3
CH3
CH3
CH3
HO

Cholesterol (animal)



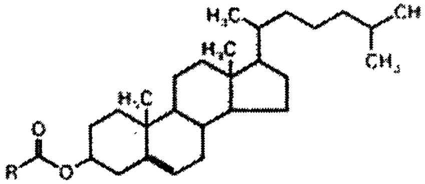
CH3
H3C
CH3
CH3
CH3
CH
HO

Ergosterol (fungal)



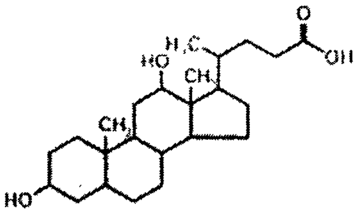
CH3
H3C
CH3
CH3
CH3
CH3
HO

Stigmasterol (plant)



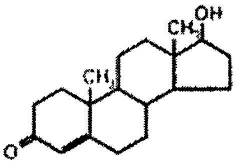
H3C
CH3
H3C
CH3
H3C
O
R
O

Cholesteryl ester

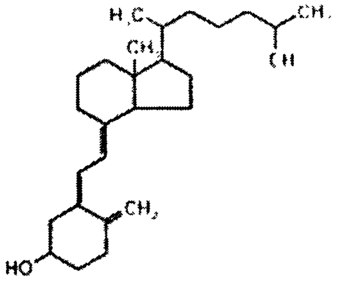


O
H3C
HO
CH3
OH
CH3
HO

Bile acid (deoxycholic acid)



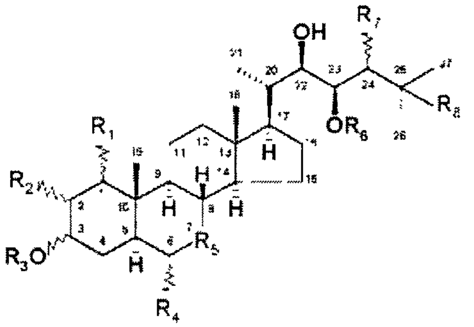
Steroid hormone (testosterone)



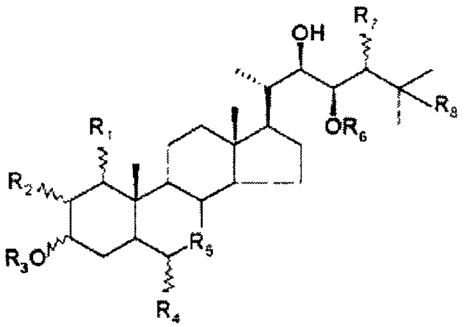
H3C
CH3
CH3
CH3
CH2
HO

Vitamin D_3

Brassinosteroids



General structural formula



Simplified general structural formula

In 1979, a novel plant growth regulating steroidal substance called brassinolide was isolated from rape (Brassica napus) pollen. Brassinosteroids are thought by some to be a new class of plant hormones. The evidences are :

- They are widely distributed in the plant kingdom.
- They have an effect at extremely low concentration.
- They have a range of effects which are different from the other classes of plant hormones.
- They can be applied to one part of the plant and transported to another where in very low amounts elicit a biological response.

Properties of Fat

Physical

- They are insoluble in water and soluble in organic solvents.
- Pure triacylglycerols are tasteless, odourless, colourless and neutral in reaction.
- They have lesser specific gravity (density) than water and therefore float in water.
- Though fats are insoluble in water, they can be broken down into minute droplets and dispersed in water. This is called emulsification.
- They contain hydrophilic colloidal particles such as proteins, carbohydrates and phospholipids which act as stabilizing agents.
- Emulsification greatly increases the surface area of the fat and this is an essential requisite for digestion of fat in the intestine.

Chemical

- Fat is hydrolysed to yield three molecules of fatty acid and one molecule of glycerol. The hydrolysis of fat is effected by alkali and enzyme.
- The process of alkali hydrolysis is called 'saponification'.
- The alkali salt of fatty acid resulting from saponification is soap
- Hydrolysis of triacylglycerol may be accomplished enzymatically through the action of lipases.
- Development of disagreeable odour and taste in fat or oil upon storage is called rancidity. Rancidity reactions may be due to hydrolysis of ester bonds (hydrolytic rancidity) or due to oxidation of unsaturated fatty acids (oxidative rancidity).
- The partial hydrolysis of the triacylglycerol to mono and diacylglycerol is called Hydrolytic rancidity. The hydrolysis is hastened by the presence of moisture, warmth and lipases present in fats or air. In fats like butter which contains a high percentage of volatile fatty acids, hydrolytic rancidity produces disagreeable odour and taste due to the liberation of the volatile butyric acid. Butter becomes rancid more easily in summer.
- The unsaturated fatty acids are oxidised at the double bonds to form peroxides, which then decompose to form aldehydes and acids of objectionable odour and taste (Oxidative rancidity).

Constants of Fats and Oils

Since fats and oils form essential nutrient of human diet, it is necessary to identify a pure fat or to determine the proportion of different types of fat or oil mixed as adulterant in edible oils and fats like butter and ghee. With an adequate knowledge of the characteristic composition of fats or oils, it is possible to identify the fat or oil under investigation. The chemical constants also give an idea about the nature of fatty acids present in fats or oils. Eventhough gas chromatographic method is available to identify and quantify the fatty acids present in fat or oil, the physical and chemical constants are still used in routine public health laboratories where such sophisticated facilities are lacking.

Physical constants

- Specific Gravity : Since different oils have different specific gravity, any variation from normal value shows mixture of oils.
- Refractive Index : Fats have definite angles of refraction. Variation from the normal value indicates adulteration of fats or oils.

- Solidification Point or Setting Point : Solidification point is the temperature at which the fat after being melted, sets back to solid or just solidifies. Each fat has a specific solidification point.

Chemical constants

- Saponification number : It is defined as milligrams of KOH required to saponify 1 gm of fat or oil. Saponification number is high for fat or oil containing low molecular weight or short chain fatty acids and vice versa. It gives a clue about the molecular weight and size of the fatty acid in the fat or oil.
- Iodine Number : It is defined as the number of grams of iodine taken up by 100 grams of fat or oil. Iodine number is a measure of the degree of unsaturation of the fatty acid. Since the quantity of the iodine absorbed by the fat or oil can be measured accurately, it is possible to calculate the relative unsaturation of fats or oil.
- Reichert-Meisel Number (R.M.number) : It is defined as the number of millilitres of 0.1 N alkali required to neutralise the soluble volatile fatty aicds contained in 5 gm of fat. It measures the volatile soluble fatty acids. It is confined to butter and coconut oil. The determination of Reichert-Meisel number is important to the food chemist because it helps to detect the adulteration in butter and ghee. Reichert-Meisel value is reduced when animal fat is used as adulterant in butter or ghee.
- Polanski Number : It is defined as the number of millilitres of 0.1 N potassium hydroxide solution required to neutralise the insoluble fatty acids (not volatile with steam distillation) obtained from 5 gm of fat.Ghee may be adulterated by the addition of insoluble, non-volatile fatty acids (by addition of animal fat). This can be tested by finding out the Polanski number
- Acetyl Number : It is defined as the amount in millilitres of potassium hydroxide solution required to neutralise the acetic acid obtained by saponification of 1 gm of fat or oil after acetylation. Some fatty acids contain hydroxyl groups. In order to determine the proportion of these, they are acetylated by means of acetic anhydride. This results in the introduction of acetyl groups in the place of free hydroxyl groups. The acetic acid in combination with fat can be determined by titration of the liberated acetic acid from acetylated fat or oil with standard alkali. Acetyl number is thus a measure of the number of hydroxyl groups present in fat or oil.

- Acid Number : It is defined as the milligram of potassium hydroxide required to neutralise the free fatty acids present in one gram of fat or oil. Acid number indicates the amount of free fatty acids present in fat or oil. The free fatty acid content increases with age of the fat or oil.

Biological Membranes

Proteins and polar lipids account for mass of biological membranes. The relative proportions of protein and lipid differ in different membranes, reflecting the diversity of biological roles. Amphipathic molecules form a lipid bilayer with the non polar region of lipids facing outward. In this lipid bilayer, globular proteins are embedded at regular intervals held by hydrophobic interactions. Some proteins protrude from one or other face of the membrane (peripheral proteins); some span its entire width (integral proteins). The individual lipid and protein subunits in a membrane form a fluid mosaic. The membrane is fluid because the interactions among lipids, between lipids and proteins are non covalent, leaving individual lipid and protein molecules free to move laterally.

One of the key functions of a membrane is to control the passage of substances across it. They are said to be selectively permeable. The different membranes of the cell have different selective permeabilities.

Common Features

- Membranes are sheetlike, just a few molecules thick and form closed boundaries between cell compartments.
- Membranes contain lipids and proteins, with small amounts of carbohydrayes linked to the lipids and proteins.
- Lipids in membranes are small with hydrophobic and hydrophilic portions. Lipid bilayers provide a barrier to the diffusion of polar molecules.
- Characteristic functions of membranes are mediated by specific proteins, serving as pumps, channels, receptors, energy transducers and enzymes.
- Membrane components associate through noncovalent interactions.
- Membranes are asymmetrical, with two sides of the membrane differing from each other.
- Lipid and protein molecules often diffuse rapidly in the plane of the membrane.

Bilayer Formation

Hydrophobic interactions provide the primary driving force for the formation of bilayers (Fig. 3.3).

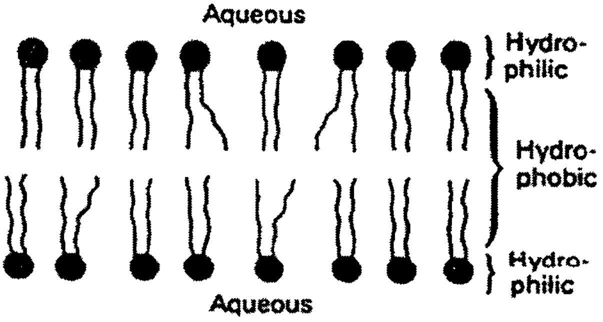

Aqueous
Hydro-philic
Hydro-phobic
Hydro-philic
Aqueous

Fig 3.3 : Arrangement of Lipid Bilayer in Cell Membrane

Saturated fatty acid chains **pack easily** and have a **higher melting temperature (Tm)**. Butter is a solid at room temperature so has a high Tm.

Unsaturated fatty acids have a **lower Tm**. Canola oil is a liquid at room temperature so has a low Tm.

Cholesterol **impedes motion** of the hydrocarbon tails making membranes **less fluid** (3.4).

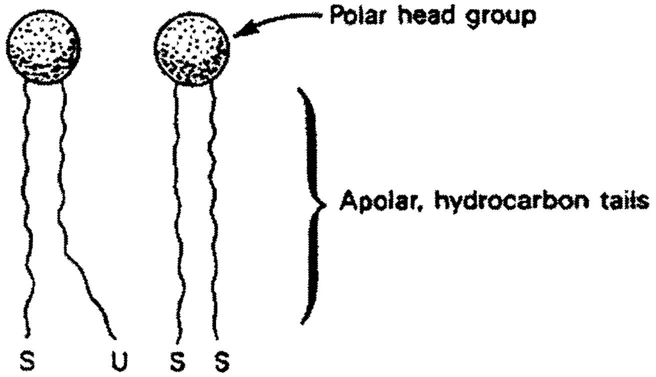

Polar head group
Apolar, hydrocarbon tails
S
U
S
S

Fig. 3.4 : Typical Structure of Head and Tail of Lipid Bilayer

The degree of saturation of the "tails" affects the stability of the membrane. Saturated fats pack more easily than unsaturated fats. A high percentage of unsaturated fats lowers the temperature at which a membrane will become rigid.

Fluid Mosaic Model

- The lipids are arranged in a bilayer, which is both a permeability barrier and solvent for integral proteins (Fig. 3.5).
- Some lipids interact with specific proteins to produce characteristic functions of the membrane.
- Lipids diffuse laterally (horizontally) rapidly but transversely (vertically) slowly. Proteins diffuse laterally.

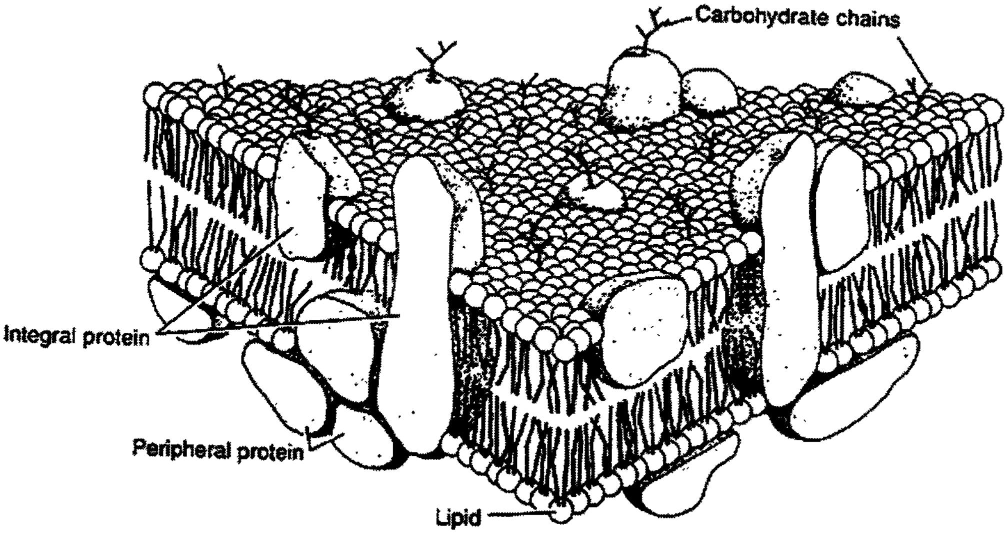
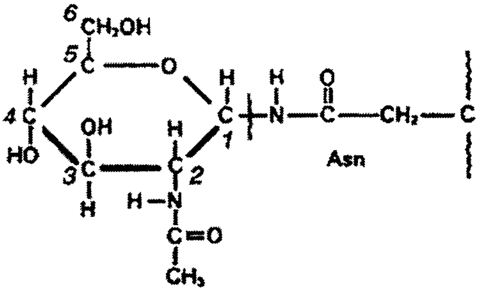
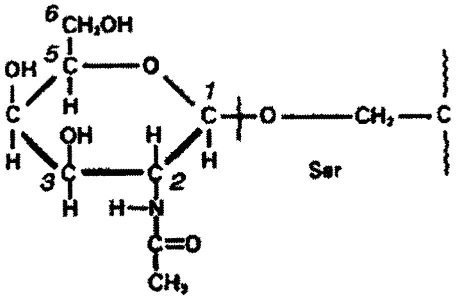


Fig. 3.5 : Fluid Mosaic Structure of Cell Membrane

Membrane Carbohydrates:

- Common monosaccharides associated with membranes are: glucose, galactose, fructose and mannose.
- Polysaccharides attached to membranes are built from monosaccharides attached to each other via glycosidic bonds.
- Hexoses can be linked from any of 5 positions on the molecule -----> possibility of numerous different structures, i.e., "*high information content*".
- Both membrane proteins and lipids can be glycosylated.
- In glycoproteins, the sugar residues are attached to nitrogen of Asn (N-linked) or via hydroxyl of a Ser or Thr (O-linked).
- Both glycoproteins and glycolipids are important in cell-cell recognition.





Membrane Asymmetry:

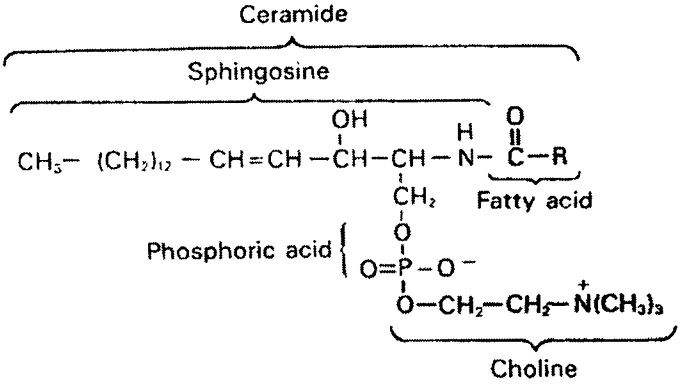
1. Membrane components are asymmetrically distributed across the bilayer.
2. Membranes are asymmetrically oriented:

   pumps *drive transport* in one direction

   receptors *bind molecules* on the outside
3. Carbohysrates are asymmetric because they are found on the outside of the surface of the cell.

Sphingolipids and Glycolipids:

Sphingomyelin (an important *spingolipid*)-
A lipid found in brain, blood cells and lung surfactant.


Ceramide
Sphingosine
Fatty acid
Phosphoric acid
Choline

Glycolipids -

- have an identical structure, except the phosphoryl choline headgroup is replaced with a monosachharide (sugar).

Peripheral Membrane Proteins:

- Are either extracellular or intracellular.
- Though associated with membranes they can be easily removed.

Extracellular Proteins:

- Protein ligands specific for cell surface receptors.
- Proteins of the extracellular matrix.

Intracellular Proteins:

- Attach to lipid anchors or to integral proteins.
- These proteins can be modified covalently by lipids ----> localizes them to specific membranes.
- Many of these modified proteins are oncoproteins --> cause cancer if they become malfunctional.

Integral Membrane Proteins:

- Span the bilayer and thus have both intra- and extracellular domains.
- The transmembrane domain is always -helical.
- They cannot be removed from the membrane without very harsh treatments.

They fall into three classes: **antigens, receptors** and **translocators**.

1. **Antigens**:

- Integral membrane proteins that are recognized by antibodies.

2. **Receptors**:

- Are required for the specific action of hormones, transmitters and growth factors.
- Three general classes of membranes receptors: growth factor receptor tyrosine kinases (RTKs), small molecule 7 transmembrane helix receptors, receptor channels.

(a) RTKs bind hormones such as insulin

They contain:

- An extracellular ligand binding domain.
- A single α-helical transmembrane domain.
- An intracellular domain which can phosphorylate tyrosine residues on proteins.

(b) Small Molecule 7 Transmembrane Helix Receptors (Fig. 3.6).

- Bind hormones such as epinephrine and glucagon.

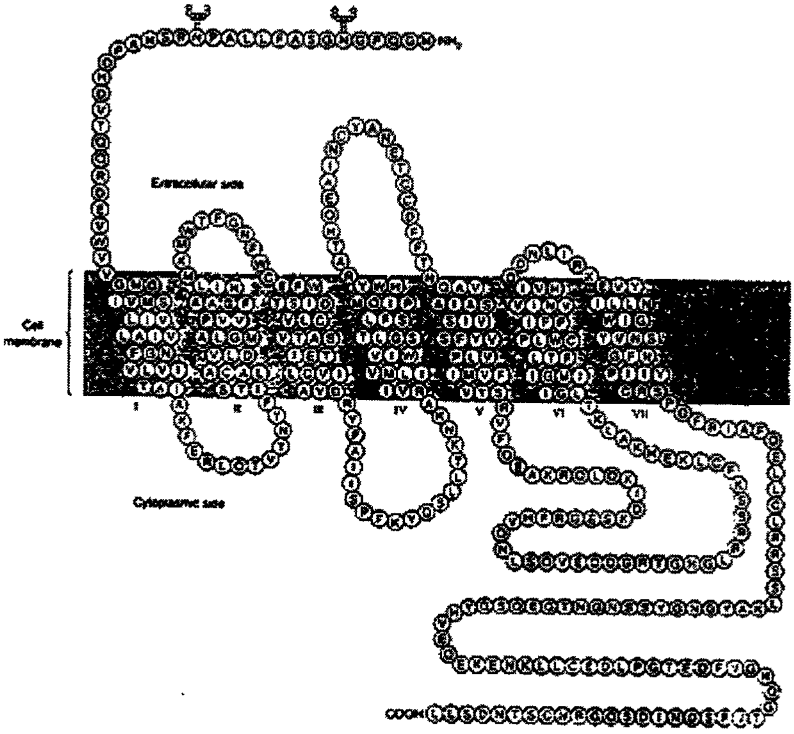

NH3
Extracellular side
Cell membrane
I
II
III
IV
V
VI
VII
Cytoplasmic side
COOH

Fig. 3.6 : Receptor proteins on out and Inside Cell Membrane.

Most are **G-Proteins**- activate other membrane proteins which bind GTP (Fig. 3.7).

G-Proteins

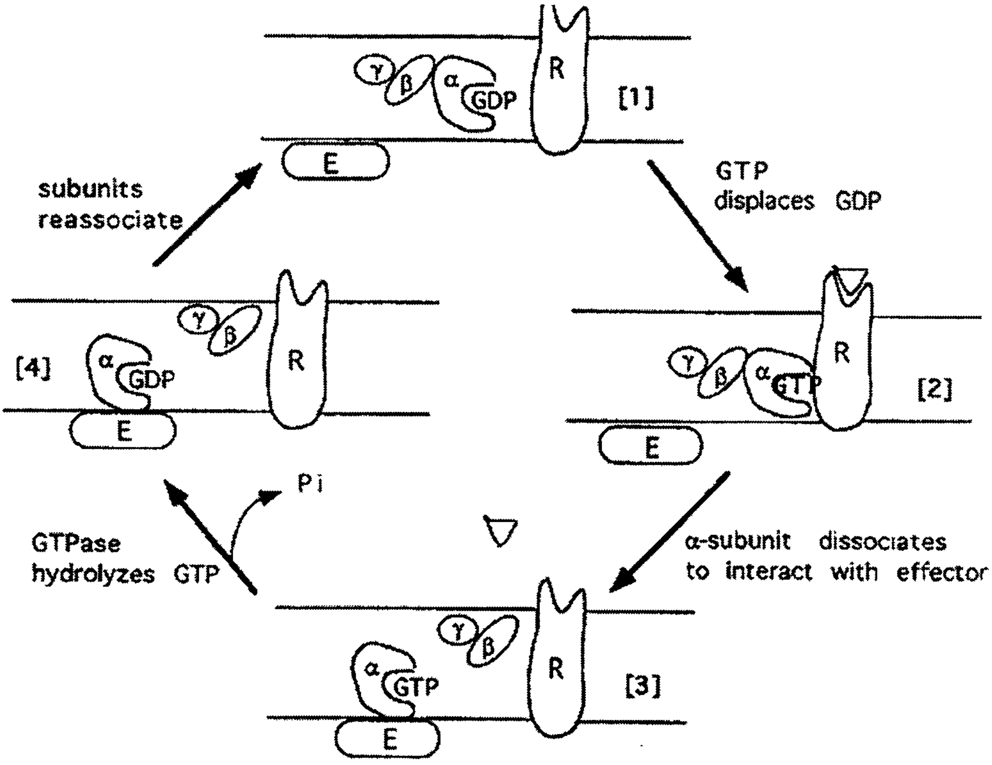

R
γ
β
α
GDP
[1]
E
GTP displaces GDP
[2]
GTP
α-subunit dissociates to interact with effector
[3]
GTPase hydrolyzes GTP
Pi
[4]
subunits reassociate

Fig. 3.7 : Schematic Presentation of G-Protein Functions in Signal Transduction

3. Transporters (Translocators):

The major function of membranes is that of a permeability barrier allowing the cell to maintain distinctly different environments between compartments.

So, cells have evolved **transport mechanisms** to move things across membranes.

Transporters may be **active** or **passive**.

Active Transport: (Fig. 3.8)

- In some cases the cell "pumps" solutes against their concentration (electrochemical) gradient.
- This is active transport because energy must be expended.
- Na^+ / K^+ ATPase (or Na^+ Pump)
- This "pump" is found in the plasma membrane of ALL *mammalian cells.*

- Its function is to pump three Na^+ ions *out* of the cell and two K^+ ions *into* the cell, at the *expense* of one ATP molecule.

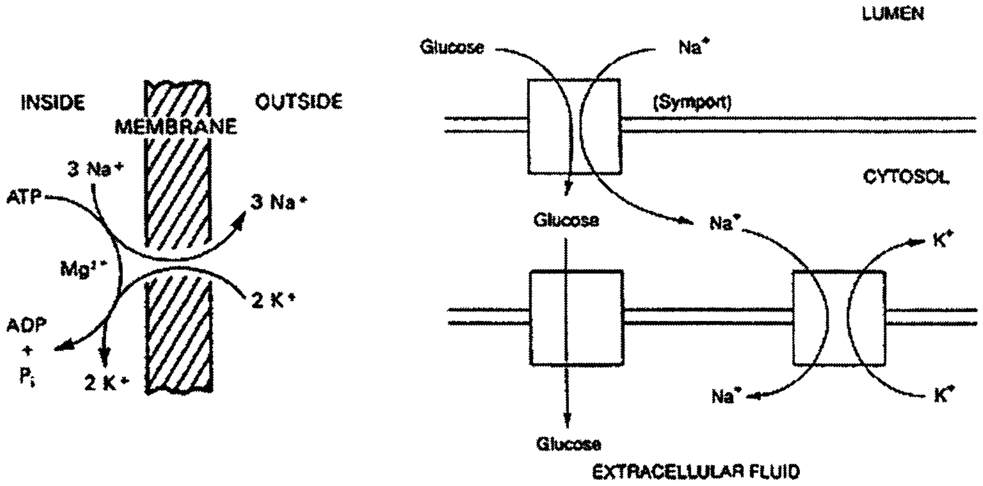

INSIDE
MEMBRANE
OUTSIDE
3 Na+
ATP
3 Na+
Mg2+
2 K+
ADP
+
Pi
2 K+
LUMEN
Glucose
Na+
(Symport)
CYTOSOL
Glucose
Na+
K+
Na+
K+
Glucose
EXTRACELLULAR FLUID

Fig. 3.8 : Active Transportation process Across the Cell Membrane

Passive Transport : Channel (*carrier-mediated*) with the Electrochemical (concentration) Gradient (Fig. 3.9).

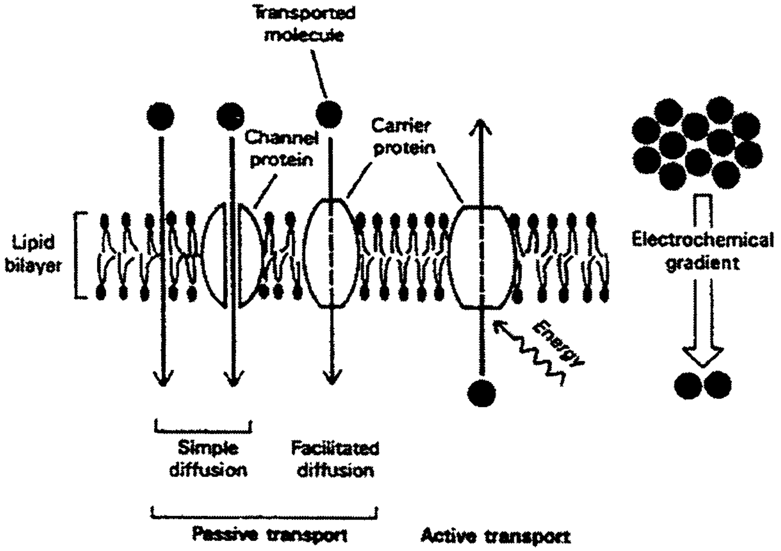

Transported molecule
Channel protein
Carrier protein
Lipid bilayer
Energy
Electrochemical gradient
Simple diffusion
Facilitated diffusion
Passive transport
Active transport

Fig. 3.9 : Passive Transportation Processes Across the Cell Membrane

QUIZ

Identify the letter of the choice that best completes the statement or answers the question.

1. What functional group is present in a triglyeride formed between glycerol and a saturated fatty acid?
   A. Carboxylic acid
   B. Alcohol
   C. Ester
   D. Hydroxyl

Answer: C

2. Which functional groups are present in an unsaturated fatty acid?
   A. Carboxylic acid and ester
   B. Carboxylic acid and alcohol
   C. Carboxylic acid and alkane
   D. Carboxylic acid and alkene

Answer: D

3. Which of the following is the molecular formula for glycerol?
   A. $CH_2OHCHOHCH_2COOH$
   B. $CH_2OHCH_2CH_2OH$
   C. $CH_2OHCHOHCH_2OH$
   D. $DH_2OHC(OH)_2CH_3$

Answer: C

4. Phospholipids are molecules that contain:
   A. Positively charged functional groups.
   B. Long water-soluble carbon chains.
   C. Cholesterol.
   D. Hydrophilic heads and hydrophobic tails.

Answer: D

5. Micelles of fatty acids in water are organized such that the ___ face the solvent and the ___ are directed toward the interior.
   A. Carboxylic acid groups; hydrocarbon chains
   B. Hydrocarbon chains; carboxylic acid groups
   C. Hydrophobic tails; hydrophilic heads
   D. None.

Answer: A

6. The lipid with the lowest energy value for human nutrition is:
   A. Cardiolipin.
   B. OlestrA.
   C. Lecithin.

D. Margarine.

Answer: B

7. Cholesterol is essential for normal membrane functions because it
   A. Cannot be made by higher organisms, e.g. mammals.
   B. Spans the thickness of the bilayer.
   C. Keeps membranes fluiD.
   D. Catalyzes lipid flip-flop in the bilayer.

Answer: C

8. According to the fluid mosaic model of cell membranes, which type of molecule spans the membrane, from its inner to outer surface?
   A. Carbohydrate.
   B. Hydrocarbon tails.
   C. PhospholipiD.
   D. Protein.

Answer: D

9. What are the membrane structures that function in active transport?
   A. Peripheral proteins.
   B. Carbohydrates.
   C. Integral proteins.
   D. Hydrophobic molecules.

Answer: C

10. All of the following are found in membranes except:
    A. Nucleic acids.
    B. Phospholipids.
    C. Glycoproteins.
    D. Glycolipids.

Answer: A

11. The membrane proteins that catalyze active transport reactions differ from soluble enzymes in that
    A. They do not enhance the rates of reaction.
    B. The product(s) of the reaction move in a specific direction.
    C. The substrate(s) of the reaction are all outside the cell.
    D. They are not specifiC.

Answer: B

12. Which of the following is not an example of a lipid found in lipid-linked proteins?
    A. Farnesyl groups.
    B. Palmitic aciD.
    C. Myristic aciD.
    D. Stearic aciD.

Answer: D

13. Which of the following molecules cannot move directly through the membrane by simple diffusion?

A. O_2
B. N_2
C. H_2O
D. CO_2

Answer: C

14. The facilitated diffusion of glucose into erythrocytes uses a mechanism called

A. Active transport
B. Antiport
C. Symport
D. Uniport

Answer: D

15. The rate-limiting step in lactose transport (in E. coli) is

A. Binding of a H+ outside the cell
B. Binding of lactose outside the cell
C. Conformational change in the permease
D. Dissociation of lactose inside the cell

Answer: C

16. The outward-facing conformation of E. coli lactose permease has a:

A. High pKa for the active site Glu residue
B. Low affinity for lactose
C. Low pKa for the active site Arg residue
D. None

Answer: A

17. The active transport of Na+ and K+ by the membrane Na+-K+ pump uses energy from:

A. Lhe membrane potential, Y.
B. ATP hydrolysis to ADP and Pi.
C. ATP hydrolysis to AMP and PPi.
D. Symport (or counter-transport) of Cl-.

Answer: B

18. An intermediate in the Na+-K+ pump transport cycle is

A. A phosphorylated Asp residue, E1~P.
B. An enzyme-adenylate complex, E~AMP.
C. An enzyme-PPi complex, E~PPi.
D. A protonated Glu residue, E-COOH

Answer: A

19. Which of the following statements about lipids is false?

A. The lipids found in biological systems are either hydrophobic or amphipathic.
B. Lipids represent highly reduced forms of carbon.
C. Lipids are highly soluble in water.
D. Upon oxidation in metabolism, lipids yield large amounts of energy.

Answer: C

20. Fatty acids that are saturated:
A. have an even number of carbon atoms
B. have single bonds between all carbon atoms
C. have an odd number of carbon atoms
D. have double bonds between some carbon atoms

Answer: B

21. Which of the following two fatty acids are the most common saturated fatty acids in nature?
A. lauric acid (dodecanoic acid) and stearic acid (octadecanoic acid)
B. myristic acid (tetradecanoic acid) and palmitic acid (hexadecanoic acid)
C. steric acid (octadecanoic acid) and arachidic acid (eicosanoic acid)
D. stearic acid (octadecanoic acid) and palmitic acid (hexadecanoic acid)

Answer: D

22. Which of the following statements about fatty acids is true?
A. The double bonds found in fatty acids are nearly always in the cis configuration.
B. Saturated fatty acid chains can pack closely together.
C. Unsaturated fatty acids produce flexible, fluid arrays because they cannot pack closely together.
D. All of the above.

Answer: D

23. Triacylglycerols are:
A. the fatty acids found only in microorganisms
B. fatty acids with highly oxidized carbons
C. a major energy reserve for animals
D. found only in animals

Answer: C

24. While many diets call for reduced intake levels of cholesterol, the plant sterols such as stigmasterol, which are very similar in structure to cholesterol, are not restricted. One of the reasons for the lack of strictures on plant sterols is:
A. The plant sterols are very different in solubility than cholesterol.
B. The plant sterols are poorly absorbed by intestinal mucosal cells.

C. The plant sterols are always present as part of the plant cell membranes and are not free in solution like cholesterol.

D. The plant sterols are readily broken down by digestive enzymes, while cholesterol is not.

Answer: B

25. The glycosphingolipids, which are important components of muscle and nerve cells, are distinguished by having:

A. one or more sugar residues in beta-glycosidic linkage at the 1-hydroxyl position of a ceramide

B. a diphosphatidylglycerol moiety attached to the sugar residues

C. phosphatidylcholine as one of the components of the glycerophospholipid structure

D. an ether linkage instead of the acyl group at the C-1 of glycerol

Answer: A

26. The primary function of the dolichyl phosphates is to:

A. serve as intermediates for cholesterol biosynthesis

B. to carry carbohydrate units in the biosynthesis of glycoproteins

C. form part of the water-insoluble waxes

D. form ether linkages to fatty acids in glycerophospholipids

Answer: B

27. A 1,2-diacylglycerol that has a phosphate group esterified at carbon atom 3 of the glycerol is a:

A. cardiolipin B. tristearin

C. sphingomyelin D. glycerophospholipid

Answer: D

28. In experiments designed to explore whether there is protein transverse asymmetry in membranes, it was shown that when trypsin was used to treat intact erythrocytes, the carbohydrate groups of the transmembrane protein glycophorin were released from the N-terminus of this protein (as glycopeptides). This was taken as evidence that the N-terminus of glycophorin must be on the exterior side of the membrane because:

A. Trypsin is too large to get inside the cell to attack interior parts of glycophorin.

B. Trypsin only attacks carbohydrate residues and not proteins.

C. Trypsin only attacks proteins when they are present in membranes.

D. Trypsin only attacks N-terminal regions of proteins and never near the C terminus.

Answer: A

29. The term "phase transition" as applied to lipid bilayers involves the conversion of a gel to a liquid crystalline phase. The Tm or transition temperature would be decreased by:

A. an increase in the average chain length of the component phospholipids
B. an increase in the degree of unsaturation of the component phospholipids
C. a decrease in the average chain length of the phospholipids
D. both b and c

Answer: D.

30. The protein bacteriorhodopsin has seven transmembrane segments. Based on your general knowledge of protein structure, you would anticipate that these transmembrane segments would consist primarily of:
A. beta-pleated sheets with alternating hydrophilic and hydrophobic residues.
B. beta-pleated sheets with primarily hydrophobic residues.
C. a mix of beta-pleated sheets and alpha-helices.
D. primarily alpha-helical sequences composed of hydrophobic residues.

Answer: D

31. In typical eukaryotic lipid bilayer membranes:
A. The long hydrocarbon tails of the component phospholipids are oriented toward the outside of the membrane.
B. The long hydrocarbon tails of the component phospholipids are oriented towards the interior of the membrane.
C. Proteins embedded in the membrane can readily flip from one side of the membrane to the other.
D. Proteins on the exterior surface of the membrane are connected by a cytoskeleton.

Answer: B

32. When lipids in bilayer are heated, they undergo phase transitions at specific melting temperatures. These melting points:
A. decrease with increasing chain length of the component lipids
B. increase with increasing chain length of the component lipids
C. are independent of the chain length of the component lipids
D. require specific proteins termed "flippases" to show a defined melting point

Answer: B

33. Which of these are among the three types of transmembrane transport?
A. passive diffusion B. facilitated diffusion
C. passive transport D. a and c

Answer: D

34. The cell membrane allows some charged atoms and molecules to diffuse through them. What is the gradient that goes to 0 (zero) when charged particles are at equilibrium across the membrane?

A. concentration gradient	B. electrochemical gradient
C. electrochemical potential	D. Faraday's constant

Answer: B

35. What is the main difference between passive diffusion and facilitated diffusion?
    - A. Facilitated diffusion uses ATP for energy, but passive diffusion does not.
    - B. Facilitated diffusion uses protein channels, but passive diffusion does not.
    - C. Passive diffusion needs a change in free energy across the membrane, but facilitated diffusion does not.
    - D. Passive diffusion exhibits saturation kinetics, but facilitated diffusion does not.

Answer: B

36. Which of the following are examples of facilitated diffusion?
    - A. transport of H+ in mitochondria to increase the energy of the cell
    - B. transport of glucose across erythrocyte cell wall
    - C. transport of chloride ions across cell membrane in erythrocytes
    - D. B and C

Answer: D

37. What is the most common molecule involved in driving active transport?

A. Proton	B. ATP
C. NADH	D. Chloride

Answer: B

38. Transport proteins are always found as:

A. cytoplasmic complexes	B. membrane-associated complexes
C. transmembrane channels	D. having high kinetic energy

Answer: C

39. If an active transport protein is mutated by scientists to decouple the hydrolysis of ATP from the transport of the transported molecule, which of the following will likely happen?
    - A. The transport molecule will become a facilitated diffusion protein.
    - B. The cell will die.
    - C. The transport molecule will stop working, but the cell will survive.
    - D. A, B, and C are all possible.

Answer: D

40. What is the function of the chloride-bicarbonate transport protein in erythrocytes?

A. It transports chloride ions to the nucleus.
B. It exchanges chloride for bicarbonate across the cell membrane.
C. It brings CO2 away from the respiring tissues to the lungs.
D. A and B

Answer: B

41. Of the following, which is the most direct way to shut down the transport of Na+ and K+ across animal cell membranes?
A. administering large amounts of an ATP non-hydrolysable analog to stop ATP binding
B. feeding the cells large amounts of radioactive glucose
C. giving the cells a dose of the toxin ouabain
D. none of the above

Answer: C

42. In humans, what is a common side effect of inhibiting Na+/K+ transport in the cells of the heart and blood vessels?
A. high blood pressure
B. low blood pressure
C. bipolar disorder
D. obesity

Answer: A

43. When a muscle contracts, what is happening to the Ca++ levels inside and outside the cell?
A. High cytosolic Ca++ amounts are released into the extracellular spaces.
B. Ion channels open to allow extracellular Ca++ to flow into the cell.
C. Ca++ in the nucleus is released into the cytoplasm and this triggers the contraction.
D. The Ca++ ions attack the stoma and cause it to contract the muscle.

Answer: B

44. What important process must take place so that muscle cells can contract?
A. All the ATP must be consumeD.
B. Excess cytoplasmic Ca++ must be pumped out.
C. The Na+/K+ pump must be reactivateD.
D. A and C

Answer: B

45. Across which cell membranes do you find the highest pH gradient in the body?
A. across axons in the brain
B. across muscle cell membranes during strenuous exercise
C. in the stomach across gastric mucosal cell membranes
D. across the membrane of osteoclasts during breakdown of bone

Answer: C

46. Which of the following is a pore-forming toxin?

A. colicin Ia B. a hemolysin
C. aerolysin D. all of the above

Answer: D

47. Gap junctions allow which of the following processes to occur?
A. transport of minerals and nutrients between adjacent cells
B. protection against cell death if an adjacent neighbor cell dies
C. movement of cells between adjacent sites
D. A and B

Answer: D

48. Gramicidin forms a(n) ________ in cell membranes?
A. coiled coil B. antiparallel sequential helix
C. antiparallel double helix D. parallel sequential helix

Answer: B

49. Which of the following statements concerning facilitated diffusion is false?
A. Net diffusion occurs only in the thermodynamically favored direction.
B. Proteins that participate in facilitated diffusion display a measurable affinity and specificity for the transported solute.
C. Transport rates for biomolecules are lower with facilitated diffusion than passive diffusion.
D. Facilitated diffusion displays saturation behavior.

Answer: C

50. In the sodium pump:
A. 2 Na+ are pumped out while 3 K+ ions are pumped in
B. 3 Na+ ions are pumped out while 2 K+ ions are pumped in
C. 2 Na+ ions are pumped in while 3K+ ions are pumped out
D. 3 Na+ ions are pumped in while 2 K+ ions are pumped out

Answer: B

51. The calcium pump consists of the following characteristics except:
A. 10 transmembrane helical segments
B. an ATP-binding domain
C. a phosphorylation site
D. one transmembrane segment, glycosylated at the carboxy terminus

Answer: D

52. The pH gradient across the gastric mucosa is maintained by:
A. passive diffusion B. facilitated diffusion
C. active transport D. all of the above

Answer: C

53. The E2-P state of the sodium/potassium ATPase binds which of the following?
A. sodium with high affinity B. potassium with high affinity

C. cardiac glycosides D. b and c

Answer: D

54. Multidrug resistance is conferred by a protein that:
A. operates by facilitated diffusion B. operates by active transport
C. has high substrate specificity D. a and b

Answer: B

55. One photon of light absorbed by H. Halobium results in which of the following processes?
A. the inward transport of 2 H+ B. the outward transport of 2 H+
C. the inward transport of Cl- D. both b and c

Answer: D

56. Most amino acid and sugar transport systems operate as:
A. symports utilizing Na+ gradients
B. antiports utilizing Na+ gradients
C. symports utilizing Cl- gradients
D. antiports utilizing Cl- gradients

Answer: A

57. Group translocation refers to which of the following processes?
A. A process in which a chemical modification accompanies transport
B. A process in which an ion and the other transported species move in opposite directions
C. A process in which an ion and another transported species move in the same direction
D. B and C

Answer: A

58. Which of the following features distinguish the phosphotransferase?
A. Phosphoenolpyruvate is both the phosphoryl donor and the energy source for sugar transport.
B. Phosphotransferase requires Enzyme I and HPr.
C. Enzyme I forms a reactive phosphohistidine intermediate.
D. all of the above

Answer: A

59. Which of the following statements concerning porins is false?
A. Porins are found in outer membranes of Gram negative bacteriA.
B. General porins sort molecules according to molecular size.
C. Specific porins contain binding sites for particular substrates.
D. Porins adopt alpha helical structures in transmembrane segments.

Answer: D

60. Which of the following toxins fit the helical channel model?
A. alpha hemolysin B. aerolysin
C. colicins D. antrax toxin protective agent

Answer: C

61. Melittin is a peptide toxin that forms alpha-helical aggregates in membranes. Which of the statements concerning helices is true?
    A. Polar and non-polar residues are randomly situated throughout the helix.
    B. Non-polar residues face the ion channel.
    C. Non-polar residues face the hydrophobic interior of the membrane.
    D. Polar residues face the hydrophobic interior of the membrane.

Answer: C

62. Which of the following statements is true?
    A. Channels are sensitive to membrane phase transitions, while carriers function efficiently only above a membrane phase transition.
    B. Carriers adopt a fixed orientation in a membrane.
    C. Channels are temperature-independent, while carriers function only above a membrane transition phase.
    D. A and B

Answer: C

Complete each sentence or statement.

1. ________ is the simultaneous transmembrane movement of two molecules in opposite directions through a transport protein.

Answer: Antiport

2. An area of a lipid bilayer with a distinct lipid composition and near-crystalline consistency is called a/an ________.

Answer: lipid raft

3. The movement of a lipid within one leaflet of a bilayer is called ________.

Answer: lateral diffusion

4. ________ is the fusion of an intracellular vesicle with the plasma membrane in order to release the contents of the vesicle outside the cell.

Answer: Exocytosis

5. ________ is the charge of one mole of electrons.

Answer: Faraday

6. A lipid to which carbohydrate is covalently attached is a/an ________.

Answer:glycolipid

7. An artificial vesicle consisting of a lipid bilayer surrounding an aqueous interior is a/an ________.

Answer: liposome

8. A long-chain hydrocarbon with a carboxylic acid group at one end is a/an ________.

Answer: fatty acid

9. A/An ________ is a metabolically required substance but not a part of lipid bilayer membrane.

Answer: vitamin

10. ________ is a model of biological membranes in which integral membrane proteins float and diffuse laterally in a fluid lipid layer.

Answer: Fluid mosaic model

11. A/An ________ is an amphipathic lipid containing a polar head group and an acyl group attached to a derivative of palmitate and serine.

Answer: sphingolipid

12. A membrane protein that is embedded in the lipid bilayer is a/an ________ protein, also called an intrinsic protein.

Answer: integral membrane or intrinsic

13. A substance released by a nerve cell to alter the activity of a target cell is a/an ________.

Answer: neurotransmitter

14. A/An ________ is a lipid constructed from 5-carbon units with an isoprene skeleton.

Answer: isoprenoid

15. ________ is a lipid in which three fatty acids are esterified to a glycerol backbone.

Answer:Triacylglycerol

16. Myelin sheath is the multilayer coating of ________-rich membranes that insulates a mammalian neuron.

Answer: sphingomyelin

17. ________ is the movement of a lipid from one leaflet of a bilayer to the other.

Answer: Transverse diffusion or Flip-flop

18. An enzyme that catalyzes the movement of a lipid from one bilayer leaflet to another is ________, also called a flippase.

Answer: translocase

19. ________ is the difference in electrical charge across a membrane.

Answer: Membrane potential

20. The thermodynamically spontaneous protein-mediated transmembrane movement of a substance from high to low concentration is called ________.

Answer: passive transport

21. ________ is the simultaneous transmembrane movement of two molecules in the same direction through a transport protein.

Answer: Symport

22. The momentary reversal of membrane potential that occurs during transmission of a nerve impulse is called ________.

Answer:action potential or depolarization

23. A/An ________ is any member of a broad class of macromolecules that are largely or wholly hydrophobic and therefore tend to be insoluble in water but soluble in organic solvents.

Answer: lipid

24. ________ is an amphipathic lipid in which two fatty acyl groups and a polar phosphate derivative are attached to a glycerol backbone.

Answer: Glycerophospholipid

25. A/An ________ is loaded with neurotransmitters to be released from the end of an axon.

Answer: synaptic vesicle

Indicate whether the sentence or statement is true or false

1. Diffusion is an active process that requires a cell to expend a great deal of energy.

Answer : F

2. During diffusion, molecules diffuse from a region where their concentration is low to a region where their concentration is higher until they are evenly dispersed.

Answer : F

3. A cell placed in a strong salt solution would probably burst because of an increase in osmotic pressure.

Answer : F

4. When the concentration of solutes outside the cell is equal to the concentration of solutes inside the cell, the cell solution is isotonic relative to its environment.

Answer : T

5. Diffusion occurs only in living systems.

Answer : F

6. The transport of specific particles through a membrane by carrier proteins is known as facilitated diffusion.

Answer : T

7. Ion channels are usually able to transport only one type of ion.

Answer : T

8. Facilitated diffusion moves molecules and ions in one direction only, while active transport moves them in two directions.

Answer :F

9. In active transport, energy is required to move a substance across a cell membrane.

Answer :T

10. Both the sodium-potassium pump and the proton pump require energy to move particles across the cell membrane.

Answer : T

11. The sodium-potassium pump moves sodium and potassium ions against the concentration gradient.

Answer : T

12. Exocytosis helps the cell rid itself of wastes.

Answer : T

13. During the process of exocytosis, the cell membrane extends to engulf substances that are too big to pass through the cell membrane.

Answer : F

14. Active transport systems are a form of cell transport that requires energy from molecules of ATP.

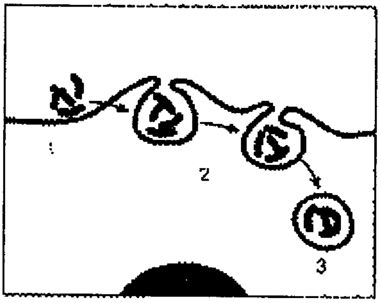
Answer : T

15. Active transport allows a cell to stockpile substances in far greater concentrations than they occur outside the cell.

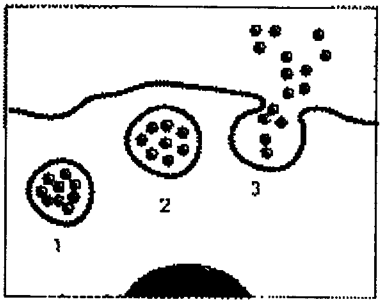
Answer : T

16. The transport of food into cells involves the action of the sodium-potassium pump and coupled channels.

Answer : T



A



B

17. Refer to the illustration above A and B. The process shown in figure "B" is called exocytosis.

Answer : T

18. Refer to the illustration above. Cells often trap extracellular particles and fluid. This is shown in figure A.

Answer : T

19. The process in which an amoeba engulfs its prey and takes it in is known as phagocytosis.

Answer : T

20. Characteristic functions of membranes are mediated by specific proteins, serving as pumps, channels, receptors, energy transducers and enzymes.

Answer : T

Chapter 4

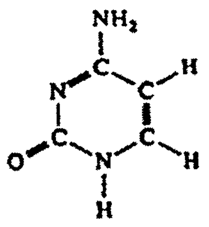
Nucleic Acids

Nucleic Acid Building Blocks

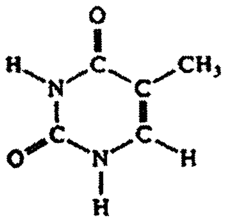
Nucleic acids are composed of nucleotide monomers, which themselves are built from a phosphate group, a sugar, and a nitrogenous base. The bases are of two types, pyrimidines (single ringed) and purines (double ringed).

The Bases

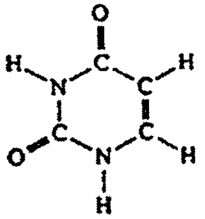
Pyrimidines:



Cytosine

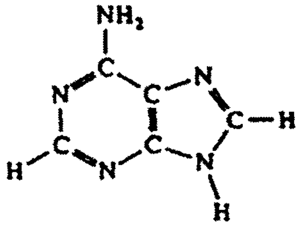


Thymine

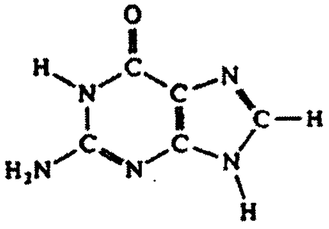


Uracil

Purines:

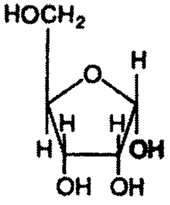


Adenine



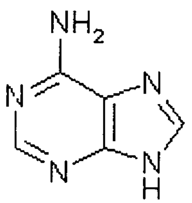
Guanine

The Ribose Sugar

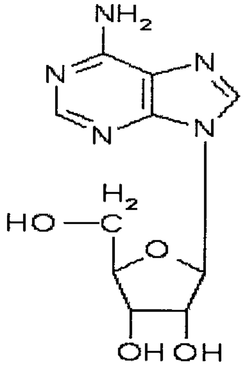


Formation of Nucleoside and Nucleotides

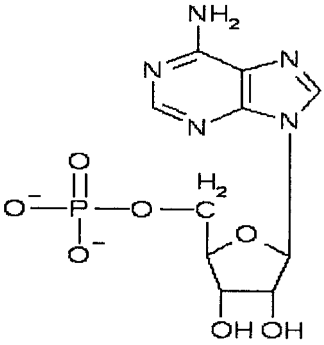
Nucleosides and nucleotides are combinations of a base with a sugar. A nucleoside is an *N*-glycoside formed between a base and a sugar (usually ribose or deoxyribose). A nucleotide is a phosphate ester of a nucleoside. DNA nucleotides are more stable to acid hydrolysis of the glycosidic bond, which is one reason that DNA has superceded RNA as the main genetic storage molecule; it is less prone to mutation.



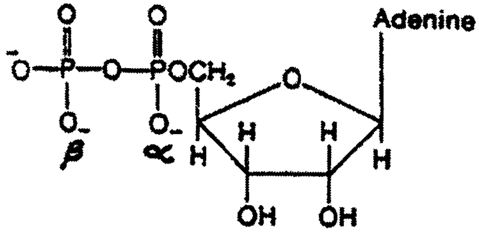
Adenine



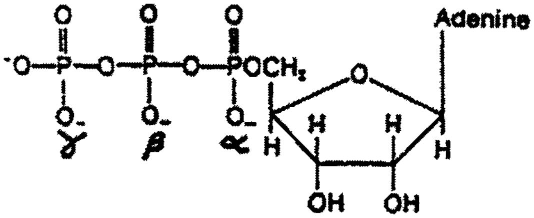
Adenosine.



Adenosine monophosphate (AMP)

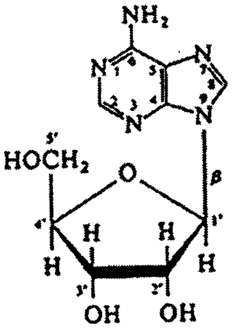


Adenosine diphosphate (ADP)

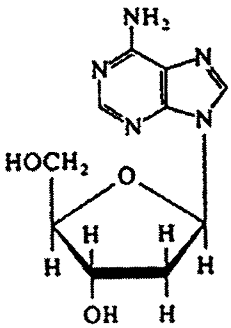


Adenosine triphosphate (ATP)

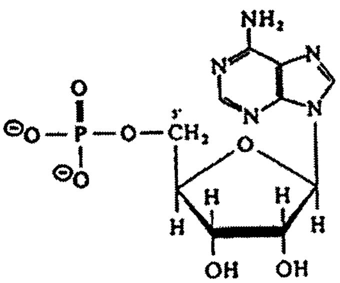
Comparison of Ribonucleoside, Deoxyribonucleoside and Ribonucleotide



Ribonucleoside



Deoxyribonucleoside



Ribonucleotide

Nucleotide Composition

Base	Ribonucleoside (Base + Ribose)	Ribonucleotide (Base + Ribose + Phos.)
Adenine (A)	Adenosine	Adenosine 5'-monophosphate (AMP)
Guanine (G)	Guanosine	Guanosine 5'-monophosphate (GMP)
Cytosine (C)	Cytidine	Cytidine 5'-monophosphate (CMP)
Uracil (U)	Uridine	Uridine 5'-monophosphate (UMP)

Types and Functions of Nucleic Acids

There are two types of nucleic acids, deoxyribonucleic acid or DNA, and ribonucleic acid or RNA. DNA stores genetic information used for the synthesis of proteins including enzymes and is found in the nucleus and mitochondria. RNA has several functions and is found in the nucleus, cytosol and mitochondria. Messenger RNA (mRNA) carries genetic information obtained from DNA to sites that translate the information into a protein. Transfer RNA (tRNA) carries activated amino acids to sites where the amino acids are linked together to form polypeptides. Ribosomal RNA (rRNA) is a structural component of ribosomes, which serve as the sites for protein synthesis. Small nuclear RNA (snRNA) is a component of small nuclear ribonucleoprotein particles. These particles process heterogeneous RNA (hnRNA, the immature form of mRNA) into mature mRNA. In some viruses, HIV, influenza, polio, RNA functions as the storage house of genetic information.

Primary Structure of Nucleic Acids

The sequence or order of the nucleotides defines the primary structure of DNA and RNA. The nucleotides of the polymer are linked by phosphodiester bonds connecting through the oxygen on the 5' carbon of one to the oxygen on the 3' carbon of another. The Oxygen and Nitrogen atoms in the backbone give DNA and RNA "polarity" (Fig. 4.1).

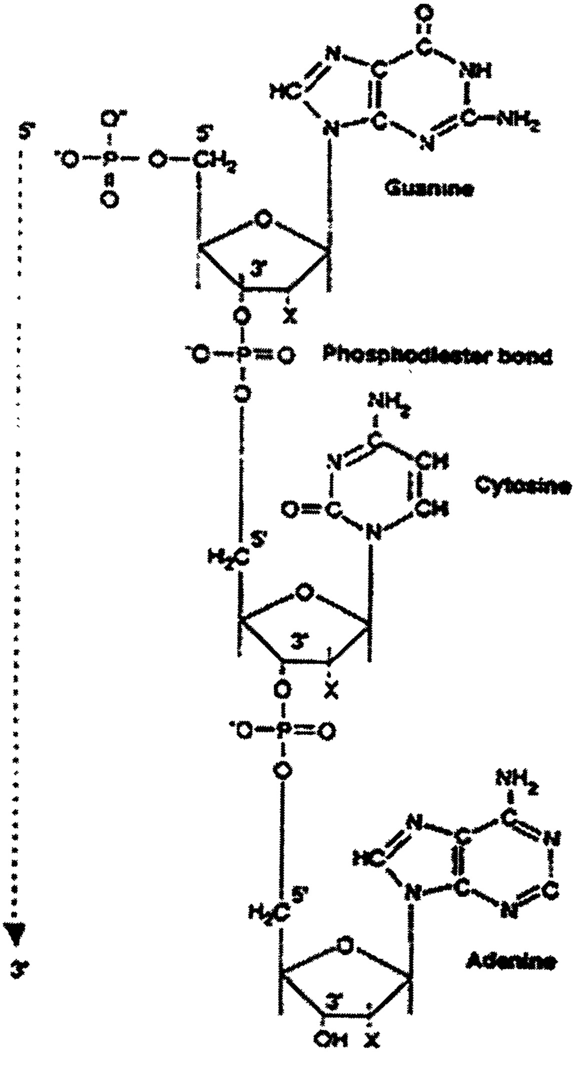

5'
3'
Guanine
Phosphodiester bond
Cytosine
Adenine

Fig. 4.1 : Nucleotides Linked Through Phosphodiester Bond

Secondary Structure of Nucleic Acids

A purine base always pairs with a pyrimidine base or more specifically Guanosine (G) with Cytosine (C) and Adenine (A) with Thymine (T) or Uracil (U) (Fig. 4.2).

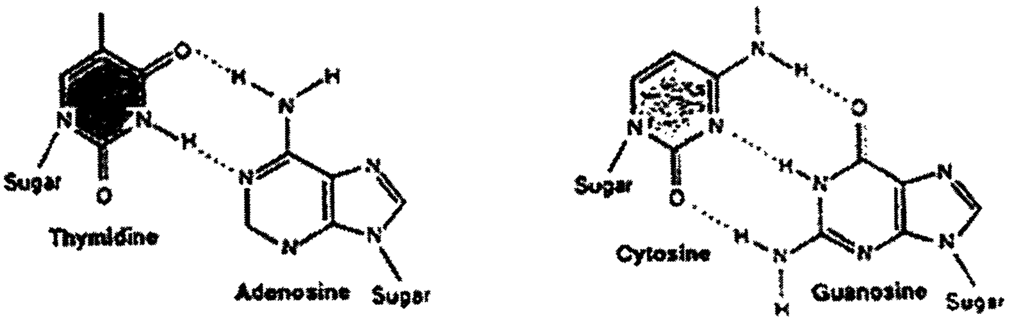

Sugar
Thymidine
Adenosine
Sugar
Sugar
Cytosine
Guanosine
Sugar

Fig. 4.2 : Base pairing, A with T (A = T) and F with C (CG ≡ C)

The G-C pair has three hydrogen bonds while the A-T pair has two hydrogen bonds.

DNA: The secondary structure of DNA consists of two polynucleotide chains wrapped around one another to form a double helix. The orientation of the helix is usually right handed with the two chains running antiparallel to one another (Fig. 4.3).

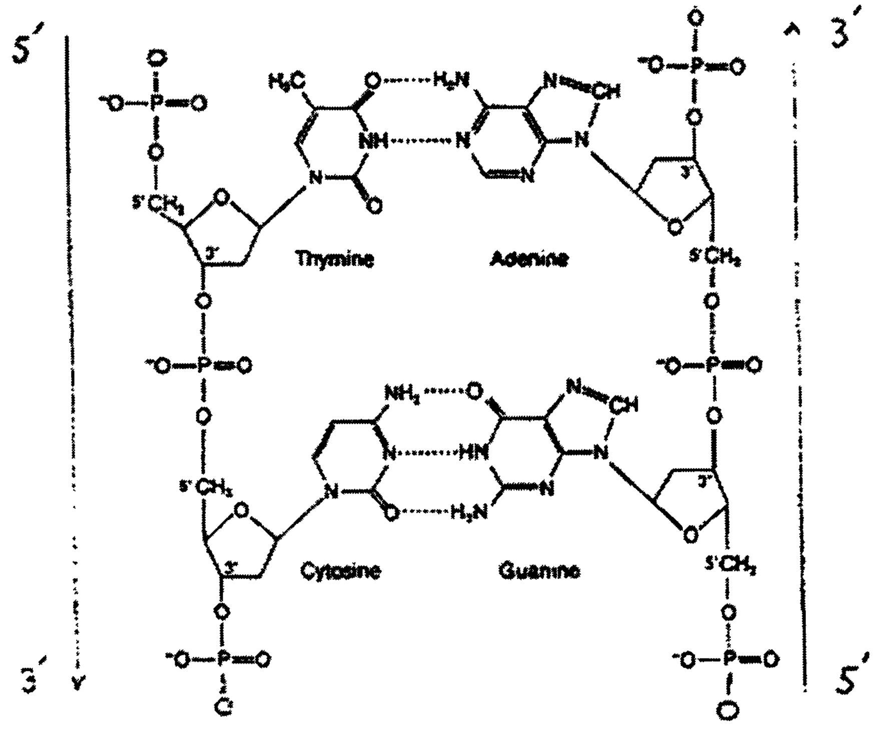

5'
3'
Thymine
Adenine
Cytosine
Guanine
3'
5'

Fig. 4.3 : Secondary Structure of DNA

Complementarity

The sequence of bases on each strand are arranged so that all of the bases on one strand pair with all of the bases on another strand, i.e. the number of guanosines always equals the number of cytosines and the number of adenines always equals the number of thymines.

There are two grooves, one major and one minor, on the double helix. Proteins and drugs interact with the functional groups on the bases that are exposed in the grooves (Fig. 4.4).

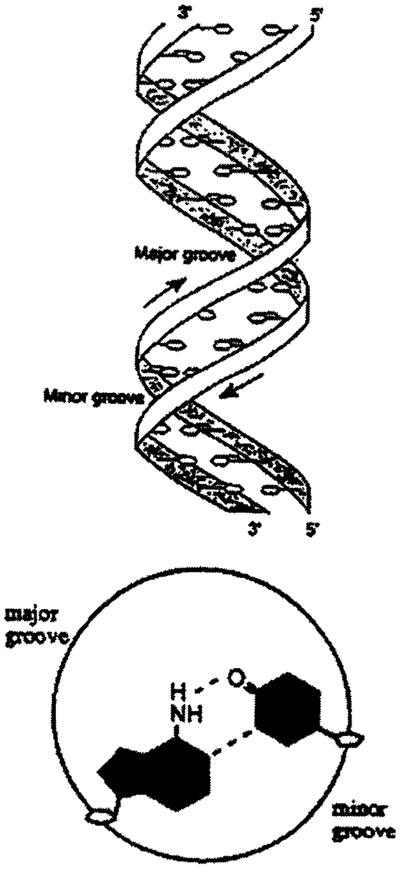
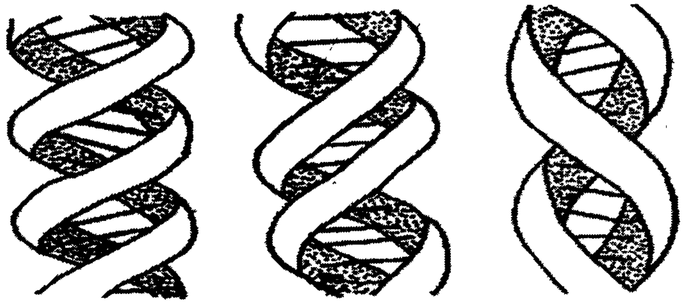


Fig. 4.4 : Grooves in Double Stranded DNA

The structural forms of DNA can differ in four aspects: the "handedness" (right or left), the length of the helix turn, the number of base pairs per turn, and the difference in size between the major and minor grooves. The most common structural form of DNA is the B-form (Fig. 4.5).

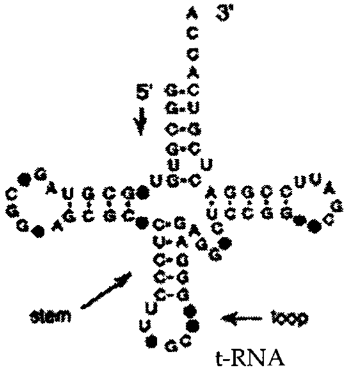


Form	A	B	Z
helical twist	right	right	left
base pairs per turn	11	10	12
occurrence	RNA, DNA	DNA	DNA

Fig. 4.5 : Different Types of Double Stranded DNA

RNA: The secondary structure of RNA consists of a single polynucleotide. RNA can fold so that base pairing occurs between complementary regions. RNA molecules often contain both single- and double-stranded regions. The strands are antiparallel and assume a helical shape. The helices are of the A-form (see above).

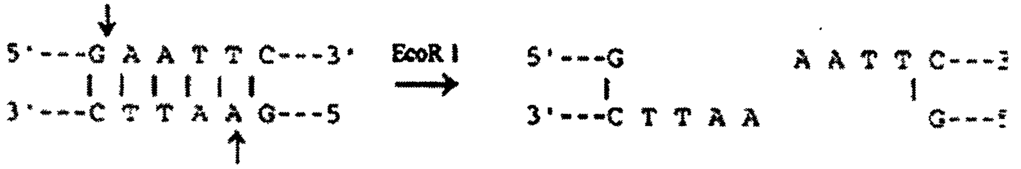
The structure of t (transfer) and r(ribosomal) RNA consists of multiple, single stranded, stem-loop structures. The stems consist of helices formed by base pairing of complementary regions within the RNA. The secondary structure of tRNA and rRNA are important for their biological functions, mRNA also assumes some degree of secondary structure but not to the same extent as tRNA and rRNA.



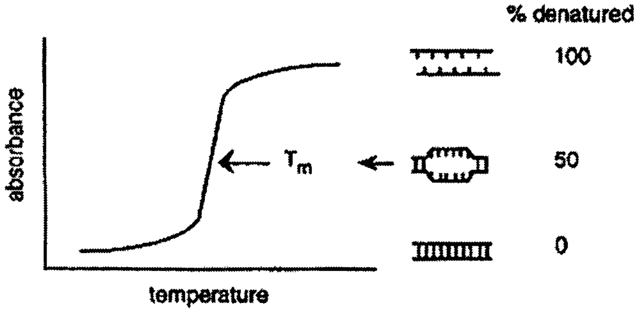
t-RNA

r-RNA

Some of the bases in DNA and RNA can be chemically modified via methylation. Enzymes, similar to proteases, called exo- and endo-nucleases can cleave RNA and DNA. Exonucleases cleave nucleic acids from the ends. Endonucleases recognize specific sequences of duplex DNA and cleave at a specific site within or near the recognized sequence. The sequences that are recognized range from four to eight base pairs in length. The resulting fragments can be joined to other fragments to create new combinations of DNA sequences.



DNA can be denatured into single strands and renatured back into a double helix. Reversible denaturation is essential for the biological processes of replication and transcription; and for molecular biological techniques such as Southern blotting and polymerase chain reactions (PCR's). There are three ways to denature DNA: enzymatically, chemically or with heat. The denaturation of DNA via heat can be followed with a spectrophotometer set to a wavelength of 260 nm. Absorption increases with increasing heat breaking the hydrogen bonds that hold the strands together, unstacking and exposing the bases. This effect is called the hyperchromic effect. The temperature at which 50% of the DNA is denatured is called the melting temperature or T_m. Because GC base pairs are held together with three hydrogen bonds (AT have only two) the higher the percentage of GC base pairs the higher the T_m required to melt the DNA.



For renaturation to take place the two strands of DNA must contact one another to initiate base pairing. Once this happens the two strands quickly reassociate along their entire length. Several things influence renaturation: complexity, DNA concentration, cation concentration and temperature. Cations such as sodium, potassium and magnesium decrease the intermolecular repulsion of the negatively charged phosphate backbones of the two DNA strands. Renaturation will only occur if the temperature is below the Tm, however if the temperature is too low the rate of renaturation will decrease. Studies have shown that most of the mammalian renatures DNA consists of repetitive sequences with only about 5% being unique sequences encoding proteins and enzymes.

Hybridization can occur between complementary strands of nucleic acids derived from different sources. The double stranded nucleic acid that forms is a heteroduplex and the extent of heteroduplexes indicates homology between the two nucleic acid sources. For example, humans and mice mix with a very small fraction of the DNA renaturing but humans and chimpanzees give greater than 98% homology. DNA and RNA can also hybridize with one another to form heteroduplexes.

The study of the replication and expression of genetic information involves a unidirectional flow from DNA to RNA to Protein:

DNA ------------->RNA------------->Protein

Study of genetics will examine these processes in relation to the diagram shown below. (Fig. 4.6)

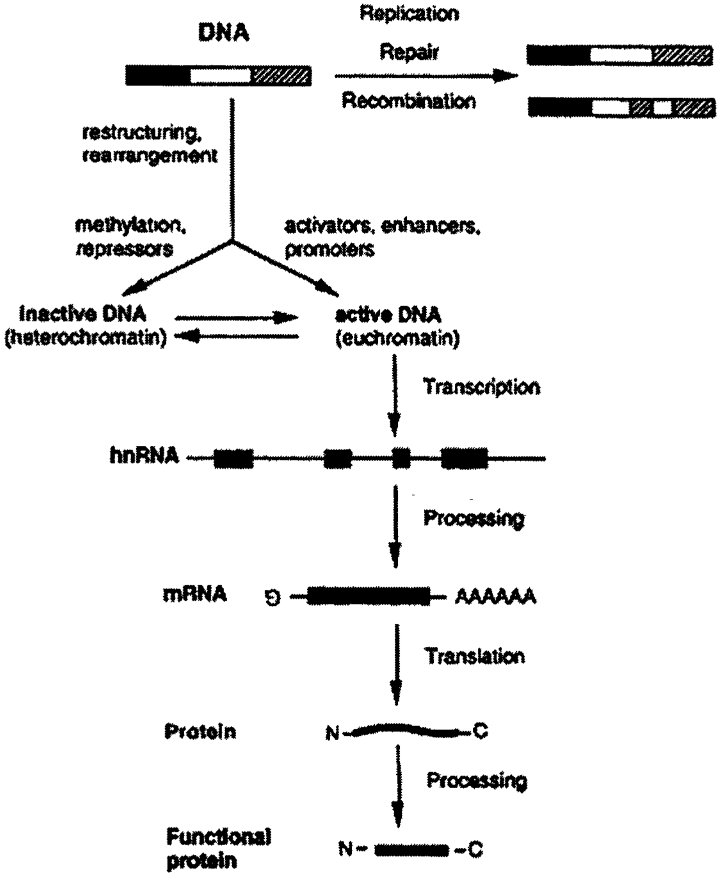

DNA
Replication
Repair
Recombination
restructuring,
rearrangement
methylation,
repressors
activators, enhancers,
promoters
inactive DNA
(heterochromatin)
active DNA
(euchromatin)
Transcription
hnRNA
Processing
mRNA
AAAAAA
Translation
Protein
N-
-C
Processing
Functional
protein
N-
-C

Fig. 4.6 : Replication and Expression of Genetic Information

DNA and Inheritance

Until the 1940s biologists argued about whether DNA or protein was the molecule of heredity.

- Nuclei observed to divide when cells reproduced themselves.
- Large amounts of both DNA & protein in the nucleus.
- Many though protein was most likely molecule of heredity.
- Proteins made of 20 different amino acids- could have more variety than DNA (made of only 4 different bases).

Pneumonia bacteria have a transforming factor.

- Pneumonia bacteria (*Streptococcus pneumoniae*) exist in 2 forms:
    - R (rough): harmless.
    - S (smooth): "wild type", causes disease.
        - The S type is coated with a polysaccharide which makes it infective and gives the colonies a smooth appearance.
- Frederick Griffiths about 1928 studied the R & S strains by injecting them into mice.
    - S injected into mice -> pneumonia -> death.
    - R injected into mice -> harmless.
    - Also, boiled S injected into mice -> harmless (bacteria killed by boiling).
    - The Griffiths did a strange experiment and got a strange result:
        - Boiled S + live R injected into mice -> pneumonia -> death.
        - This was not expected because boiled S and live R were harmless by themselves.
        - Took blood samples and found live S in the dead mice.
        - Concluded that some factor, a "transforming principle", from the dead S had converted some R bacteria into S bacteria (a genetic change).

Table 4.1 : Summary of Griffith's Experiments

Injected	Result	Live S in Blood ?
Live R	No disease	No
Live S	Death	Yes
Killed S	No disease	No
Killed S + Live R	Death	Yes

The transforming factor was found to be DNA.

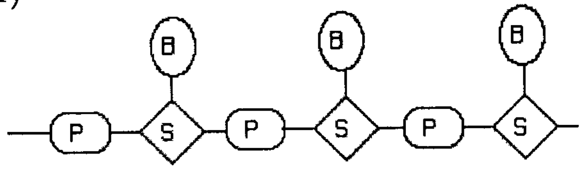
- Oswald Avery wanted to know the nature of the transforming principle.
- Spent many years purifying transforming principle from killed S bacteria.
- Finally determined it was DNA 1944.
- First clear cut evidence for hereditary role of DNA.

Bacteriophage DNA changes the hereditary functions of bacteria.

- Bacteriophage are a type of virus that attacks bacteria.
- Consist of DNA with a protein coat.
- When virus attacks bacterium its DNA is inserted, but not its protein.
- Viruses alter genetic function of bacteria so that they make virus proteins and DNA.
- Again DNA shown to have a genetic function.

By the early 1950s, it was believed that the secret of heredity was in the structure of DNA.

- Many laboratories began studying the structure of DNA, hoping to find the secret of heredity.
- The basic composition of DNA was known.
    - A sugar-phosphate backbone, with 4 nucleotide bases (A, C, G & T)



(In the diagram S = sugar; P = phosphate; B = base)

- Chargaff had measured the base composition in DNA from many species and found that always A = T and C = G.
- X-ray diffraction pictures made by Rosalind Franklin showed that the DNA structure was a helix (2 or more molecules spiraling around each other); the structure appeared to have a uniform thickness.

QUIZ

Identify the letter of the choice that best completes the statement or Answers the question.

1. The bonding between complementary base pairs is
   - A. Peptide links
   - B. Ester links
   - C. Van der Waal's forces
   - D. Hydrogen bonding

Answer: D

2. The following base pairs can produce complementary base pairing:
   - A. T - U
   - B. A - T
   - C. T - C
   - D. A - G

Answer: B

3. The sugar present in the nucleotides of DNA is:
   - A. Ribose
   - B. Glucose
   - C. Fructose
   - D. Deoxyribose

Answer: D

4. The type of reaction that forms the sugar-phosphate chains in DNA and RNA is:
   - A. substitution
   - B. condensation polymerisation
   - C. esterification
   - D. addition polymerisation

Answer: B

5. Which of the following are purine bases?
   - A. adenine and cytosine
   - B. adenine and uracil
   - C. adenine and guanine
   - D. adenine and thymine

Answer: C

6. Which of the following are pyrimidine bases?
   - A. cytosine and guanine
   - B. adenine and thymine
   - C. cytosine and thymine
   - D. cytosine and adenine

Answer: C

7. The sugars in nucleotides are:
   A. heptoses
   B. hexoses
   C. trioses
   D. pentoses

Answer: D

8. The four bases in RNA are:
   A. adenine, cytosine, thymine, uracil
   B. adenine, guanine, thymine, uracil
   C. adenine, guanine, cytosine, uracil
   D. adenine, guanine, cytosine, thymine

Answer: C

9. Intramolecular bonds in nucleotides are formed by:
   A. hydrolysis reactions
   B. condensation reactions
   C. polymerisation reactions
   D. esterification reactions

Answer: B

10. The three components of nucleotides are:
    A. a sugar, a phosphate group, a nitrogen-containing base
    B. a sugar, a phosphate group, an amino acid
    C. glucose, a phosphate group, an ester
    D. glucose, a phosphate group, a nitrogen-containing organic base

Answer: B

11. The B-DNA structure found in solution is a
    A. right-handed double helix of antiparallel chains (11 bp/turn).
    B. left-handed double helix of antiparallel chains (~10 bp/turn).
    C. right-handed double helix of antiparallel chains (~10 bp/turn).
    D. left-handed zig-zag helix of antiparallel chains (12 bp/turn).

Answer: C

12. DNA differs from RNA in the following features
    A. DNA residues are linked by 3'-->5' phosphodiester bonds; RNA is 2'-->5' linked.
    B. DNA has deoxyribose residues; RNA has ribose residues.
    C. DNA contains the A, C, G and T bases; RNA contains A, C, G, and U.
    D. All

Answer: D

13. Because DNA is a highly charged polyanion, its stability to heat denaturation ("melting"):
    A. does not depend on hydrophobic interactions.
    B. increases with increasing salt.

C. decreases with increasing salt.
D. is independent of G + C content.

Answer: B

14. The total contour length of DNA in a human cell is about
    A. 1 mm.
    B. 1 mm.
    C. 10 cm.
    D. 1 meter.

Answer: D

15. Polyacrylamide and agarose gel electrophoresis separate nucleic acids based primarily on their
    A. length.
    B. ratio of mass/charge.
    C. (G+C)/(A+T) content.
    D. organismal origin.

Answer: A

16. The number of supercoils in a covalently-closed, circular DNA can only be changed if
    A. at least one of the phosphodiester chains is cleaved.
    B. both of the phosphodiester chains are cleaved.
    C. histones are bound to the DNA.
    D. chemical reagents react with the backbone phosphates.

Answer: A

17. Thymidine
    A. is replaced by Uracil in RNA.
    B. normally forms 2 hydrogen bonds with adenosine.
    C. can participate in hydrophobic interactions due to its methyl group.
    D. All

Answer: D

18. The major and minor grooves of B-form DNA correspond to what features of A-form RNA?
    A. minor and major grooves
    B. deep and shallow grooves
    C. deoxyribose backbones
    D. phosphoribose backbones

Answer: B

19. The glycosidic bonds in DNA and RNA
    A. can be hydrolyzed by OH^-.
    B. are restricted to one of four possible orientations.
    C. connect the sugar to the base.
    D. stabilize Watson-Crick H-bonds.

Answer: C

20. In solution, the grooves of nucleic acid helices
    A. expose the H-bonding groups of the bases.
    B. expose the hydrophobic surfaces of the bases.
    C. are about equal in width.
    D. None

Answer: A

21. When an aromatic molecule intercalates into the DNA double helix, the two adjacent base pairs are separated by about ______, and the helix is unwound by about _______.
    A. 3.4Å, 36°
    B. 3.4Å, 26°
    C. 4.3Å, 26°
    D. 1.7Å, 10°

Answer: B

22. The successive mononucleotide units in DNA and RNA are:
    A. linked by hydrogen bonding through phosphodiester bridges between the 3' position of one mononucleotide and the 5' position of the next
    B. linked by hydrogen bonding between adenine and thymine and between guanine and cytosine
    C. linked covalently through phosphodiester bridges between the 3' position of one mononucleotide and the 5' position of the next
    D. linked covalently between adenine and thymine and between guanine and cytosine

Answer: C

23. The sequence of bases in the part of a complementary strand of DNA that pairs with
    5'- adenine - cytosine - adenine - guanine -3' is
    3'- ______ - ______ - ______ - ______ -5'
    (i) (ii) (iii) (iv)
    A. cytosine - adenine - cytosine - thymine
    B. guanine - thymine - thymine - cytosine
    C. thymine - adenine - thymine - cytosine
    D. thymine - guanine - thymine - cytosine

Answer: D

24. In DNA, the phosphodiester linkages between the adjacent nucleotides are between:
    A. the 3' and 4' positions of the deoxyribose units
    B. the 4' and 5' positions of the deoxyribose units
    C. the 1' and 5' positions of the deoxyribose units

D. the 3' and 5' positions of the deoxyribose units

Answer: D

25. In double-stranded DNA, hydrogen bonding between the bases on the two strands typically occurs between:
A. adenine and guanine
B. adenine and thymine
C. adenine and cytosine
D. adenine and uracil

Answer: B

26. Both purines and pyrimidines can exist in different tautomeric forms. The forms typically found in double-stranded DNA are:
A. uracil = keto; adenine = keto
B. guanine = keto; cytosine = enol
C. thymine = keto; guanine = keto
D. guanine = enol; thymine = keto

Answer: C

27. In the nucleotides found in nucleic acids, the linkage between the bases and the sugar involve:
A. N-1 of pyrimidines and C-1 of the sugar
B. N-3 of pyrimidines and C-1 of the sugar
C. N-1 of purines and C-5 of the sugar
D. N-9 of purines and C-2 of the sugar

Answer: A

28. The process of "transcription" in eukaryotes involves the synthesis of:
A. an RNA copy of a DNA sequence by an RNA polymerase
B. a DNA copy of an RNA sequence by an RNA polymerase
C. an RNA copy of another RNA by an RNA polymerase
D. a DNA copy of a DNA sequence by a DNA polymerase

Answer: A

29. Transfer RNAs are involved in:
A. carrying nucleotides to the ribosome for messenger RNA synthesis
B. carrying amino acids to the ribosome for protein synthesis
C. carrying amino acids to peroxisomes for degradation
D. carrying glucose residues to the Golgi apparatus for polysaccharide synthesis

Answer: B

30. There are fundamental chemical differences between DNA and RNA, such as:
A. The nucleotides in RNA are linked by 3'-5' phosphodiester bridges while those in DNA involve 2'-5' bridges.
B. The nucleotides in RNA include uracil, while DNA has thymine.

C. The sugars in the nucleotides of RNA are hexoses, while they are the pentose deoxyribose in DNA.
D. Adenine is found in DNA but not in RNA.

Answer: B

31. DNA is much more stable to alkaline hydrolysis than RNA because:
A. DNA is usually protected by proteins, while RNA is not.
B. There are many more RNA digesting enzymes present in cells than DNA digesting enzymes.
C. The 2'-OH group of the ribose in RNA can assist hydrolysis of 3'-5' phosphodiester bridges, but DNA lacks the 2'-OH group.
D. The uracil found in RNA but not DNA aids in the hydrolysis by alkali.

Answer:C

32. The difference between the sugars in DNA and RNA is:
A. In RNA the sugar residue is neuraminic acid, while the sugar residue in DNA is deoxy-neuraminic aciD.
B. In DNA the sugar residue is deoxy-adenine, whereas in RNA it is adenine.
C. In RNA the sugar residue is a reducing sugar, whereas in DNA it is not.
D. In DNA the sugar is 2-deoxy-D-ribose, whereas in RNA it is D-ribose.

Answer: D

33. How many hydrogen bonds are formed between the complementary bases G:C and A:T, respectively?
A. 2, 3
B. 3, 2
C. 3, 4
D. 4, 3

Answer: B

34. A DNA double helix is described as a(n):
A. parallel double helix
B. antiparallel double helix
C. hydrogen-bonded double helix
D. A and C

Answer: B

35. What are the most common conformational forms of DNA?
A. A, b, and e
B. A, B, and Z
C. parallel, antiparallel, and triple helical
D. b and c

Answer: B

36. In tRNA, some unusual bases are found. Some examples of these are:
    - A. pseudouridine
    - B. inosine
    - C. methylguanosine
    - D. all of the above

Answer: D

37. This molecule is a substrate analog for:
    - A. dATP
    - B. dGTP
    - C. dUTP
    - D. dTTP

Answer: C

38. Which of the following is NOT a type of RNA in prokaryotes?
    - A. transfer RNA
    - B. messenger RNA
    - C. ribosomal RNA
    - D. small nuclear RNA

Answer: D

39. DNA stands for-
    - A. deoxynucleic acid
    - B. deoxyribonucleic acid
    - C. denatured ribonucleic acid
    - D. deoxoribonuclear acid

Answer: B

40. The process by which RNA is made from DNA:
    - A. synthesis
    - B. trbanslation
    - C. transcription
    - D. replication

Answer: C

41. Adenine always pairs with:
    - A. thymine
    - B. cytosine
    - C. guanine
    - D. ribose

Answer: A

42. The "rungs" of the DNA ladder are made of:
    - A. phosphates and hydrogen
    - B. glucose and sugars
    - C. sugars and phosphates

D. base pairs

Answer: D

43. The DNA molecule is held together by:
    A. magnetism
    B. glucose
    C. glue
    D. hydrogen bonds

Answer: D

44. The process by which DNA makes a copy of itself is called:
    A. synthesis
    B. replication
    C. transcription
    D. translation

Answer: B

45. A gene is:
    A. a segment of DNA that codes for a protein
    B. a set of homologous chromosomes
    C. a molecule within DNA
    D. a type of pants

Answer: A

46. The twisted ladder shape of DNA is called a:
    A. hydrogen twist
    B. deoxyribose flip
    C. double helix
    D. double membrane

Answer: C

47. The sugar found in DNA is:
    A. equal
    B. deoxyribose
    C. ribose
    D. glucose

Answer: B

48. Which of the following takes the genetic code to the cytoplasm:
    A. DNA
    B. deoxyribose
    C. tRNA
    D. mRNA

Answer: D

49. The three nucleotide sequence on RNA is called a:
    A. tRNA
    B. codon

C. triplet
D. gene

Answer: B

50. Three nucleotides code for:
A. 1 amino acid
B. 3 amino acids
C. 1 protein
D. 3 proteins

Answer: A

51. RNA differs from DNA in that:
A. it has a different kind of sugar
B. it is single stranded
C. it has uracil
D. all of these

Answer: D

52. DNA is called the "blueprint of life" because:
A. it is like a fingerprint
B. it has a blue color
C. it contains the plans for building an organism
D. it can relay messages to other molecules

Answer: C

53. The two men who established the structure of DNA were:
A. Frederick and Alvers
B. Watson and Crick
C. Berkely and Fry
D. Darwin and Lamarke

Answer: B

54. One side of the DNA "ladder" has the following sequence of nitrogen bases: C A T G . What is the sequence of nitrogen bases on the other side of the ladder?
A. G T A C
B. C A T C
C. A T G A
D. T A G C

Answer: A

55. If a DNA molecule were a ladder, what would make up the sides of the ladder?
A. nitrogen bases
B. sugars and acids
C. base G
D. base A

Answer: B

56. One side of the DNA "ladder" has the following sequence of nitrogen bases: A T G A. What is the sequence of nitrogen bases on the other side of the ladder?
    A. C A T G
    B. A T G A
    C. T A C T
    D. T A G A

Answer: C

57. How does DNA direct all traits in living things?
    A. by coded message
    B. by sign language
    C. by waggling its tail
    D. by speaking

Answer: A

58. What is the type of DNA formed when DNA from one organism is spliced into the DNA of another organism?
    A. recombinant DNA
    B. gene therapy
    C. RNA
    D. genetic counseling

Answer: A

59. What molecule acts as a messenger for DNA?
    A. PKU
    B. RNA
    C. ABC
    D. AIDS

Answer: B

60. Nitrogen base A can only join which nitrogen base to complete a rung in the DNA ladder?
    A. base C
    B. base G
    C. base T
    D. base A

Answer: C

61. Where are ribosomes located in a cell?
    A. in the vacuole
    B. in the nucleus
    C. in the cytoplasm
    D. in the chloroplast

Answer: C

62. What is the bringing together of two living things to produce offspring?
    A. breeding
    B. cloning
    C. grafting
    D. budding

Answer: A

63. What translates the DNA language into the protein language?
    A. electronic messenger
    B. genetic code
    C. a computer
    D. a fingerprint

Answer: B

Indicate whether the sentence or statement is true (T) or false (F).

1. An altered gene may be passed on to every cell that develops from it.

Answer: T

2. In recent years, new varieties of farm plants and animals have been engineered by manipulating their genetic instructions to produce new characteristics.

Answer: T

3. Restriction enzymes can be used to cut sequences of DNA.

Answer: T

4. The cutting, cloning, and movement of segments of DNA does not involve enzymes.

Answer: F

5. Our increased knowledge of genetics is not important to health care.

Answer: F

6. Mapping of genetic instructions in cells makes it possible to detect, and perhaps correct, defective genes that may lead to poor health.

Answer: T

7. Substances from genetically engineered organisms have increased the cost and side effects of replacing missing body chemicals.

Answer: F

8. Although all body cells are descended from a single cell and have identical genetic instructions, they may be very different because different parts of a cells instructions are used based on a cell's environment and past history.

Answer: T

9. Cell regulation is not important.

Answer: F

10. Cell regulation allows cells to respond to their environment and control and coordinate cell growth and division.

Answer: T

11. Cell regulation occurs through both changes in the activity of proteins and selective expression of individual genes.

Answer: T

12. The genetic information stored in DNA is used to direct the synthesis of the thousands of proteins required by a cell.

Answer: T

13. Proteins are long, folded molecules, but do not have specific shapes which influence their functions.

Answer: F

14. Proteins can be made from 20 different amino acids in a specific sequence.

Answer: T

15. Offspring resemble their parents because they inherit similar genes that code for the production of proteins that form similar structures and perform similar functions.

Answer: T

Complete each sentence or statement.

1. The ________ is the narrower of the two grooves on a DNA double helix.

Answer: minor groove

2. ________ lines run in opposite directions.

Answer: Antiparallel

3. The sequence of three nucleotides in DNA or RNA that specifies a single amino acid is a/an ________.

Answer: codon

4. A/An ________ is a linear stretch of genomic DNA that has been fully sequenced.

Answer: contig

5. A/An ________ organism stably expresses a foreign gene.

Answer: transgenic

6. Oligonucleotide is a polynucleotide consisting of a few nucleotide ________.

Answer: residues

7. A/An ________ is a compound consisting of a nitrogenous base linked to a five-carbon sugar (ribose or deoxyribose).

Answer: nucleoside

8. Blunt ends are the fully base-paired ends of a DNA fragment that are generated by a/an ________ that cuts both strands at the same point.

Answer: restriction endonuclease or restriction enzyme

9. ________ is an enzyme that catalyzes the formation of a phosphodiester bond to join two DNA strands.

Answer: DNA ligase

10. A gene with no assigned function is called a/an ________.

Answer: orphan gene

11. ________ is a technique in which a cloned gene is mutated in a specific manner.

Answer: Site-directed mutagenesis

12. Expressed sequence tag (EST) is a segment of DNA that is synthesized from a/an ________ template and which, therefore, represents a portion of the genome that is expressed.

Answer: mRNA

13. A base is a purine or ________ component of a nucleoside, nucleotide, or nucleic acid.

Answer: pyrimidine

14. The linkage in which a phosphate group is esterified to two alcohol groups is called a/an ________.

Answer: phosphodiester bond

15. ________ are the small L-shaped RNAs that deliver specific amino acids to ribosomes according to the sequence of a bound mRNA.

Answer: Transfer RNA or tRNA

16. ________ is the loss of ordered structure in a polymer, such as the disruption of native conformation in an unfolded polypeptide or the unstacking of bases and separation of strands in a nucleic acid.

Answer: Denaturation

17. Genes that are related by evolution from a common ancestor are called ________ genes.

Answer: homologous

18. ________ is a nucleotide sequence variation in the genomes of two individuals from the same species.

Answer: Single-nucleotide polymorphism

19. ________ is the process by which RNA is synthesized using a DNA template, thereby transferring genetic information from the DNA to the RNA.

Answer: Transcription

20. A plasmid is a small circular DNA molecule that autonomously replicates and may be used as a/an ________.

Answer: cloning vector

21. A/An ________ is a collection of immobilized DNA segments of known sequence.

Answer: microarray

22. A segment of DNA, sometimes including genes, that can move or be copied from one position to another in a genome is called a/an ________.

Answer: transposable element or transposon

23. ________ is a technique for distinguishing cells that contain a particular feature, such as resistance to an antibiotic.

Answer: Selection

24. The unit of length used for DNA molecules is ________.

Answer: bp or kb

25. ________ is an enzyme that catalyzes synthesis of a new DNA strand using an existing strand as a template for the assembly of nucleotides.

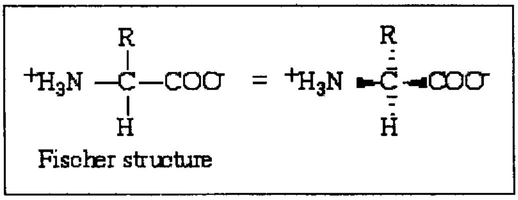
Answer: DNA polymerase

“This Page is Intentionally Left Blank”

Chapter 5

Amino Acids and Proteins

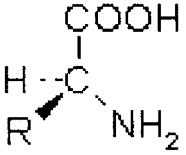
The amino acids are the building blocks for proteins - nearly all proteins studied are made from the twenty "standard" amino acids. Other amino acids are also found in proteins, but most arise by modification from the twenty after they have been incorporated in the protein. All of the standard amino acids are amino acids (except for proline, an imino acid). That is they have an amino group to the carboxyl group (they are 2-amino acids). Thus all 20 of these amino acids share the basic structure below:



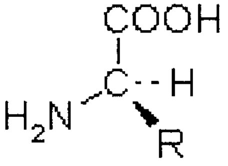
At neutral pH (pH =7) both the acid and amine groups will be ionized to give the so-called zwitterion form. There is **no** pH at which the amino acid structure will have no ionized groups.

Chemistry of Amino Acids

Amino acids are the building blocks of proteins. They all have the basic structure shown below:



L-amino acid
CO-R-N goes anticlockwise



D-amino acid
CO-R-N goes clockwise

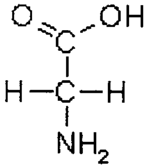
Amino acids (in general) are chiral: only the L form is found in proteins, although the D form of alanine is an important component of bacterial cell walls. All amino acids are chiral except glycine. The L/D descriptor is an old fashioned version of the S/R descriptor, and almost all amino acids are the (S)-isomer because the R group (not to be confused with an (R) descriptor!) is priority three. Only cysteine is an exception to this rule, because its R side group is $-CH_2SH$, which has higher priority than COOH. The L/D descriptors come from the 'CO-

R-N' law: if you place the hydrogen behind the chiral centre, and count round from the COOH group, to the R group, to the NH_2 group, this goes anticlockwise, and for similar reason to the R/S descriptor, this means it's the L isomer. The D-isomer goes clockwise.

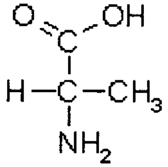
There are twenty (or so) amino acids, which we will discuss in groups based on their chemical properties.

The hydrophobic amino acids are -

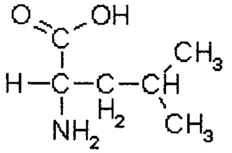
- Aliphatic : glycine (Gly, G), alanine (Ala, A), leucine (Leu, L), isoleucine (Ile, I), valine (Val, V).



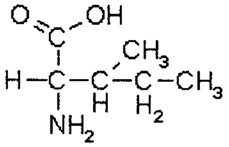
Glycine



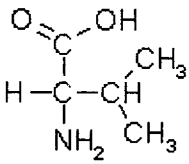
Alanine



Leucine

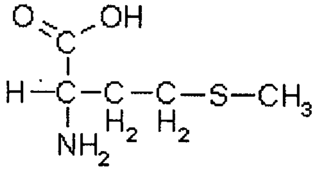


Isoleucine



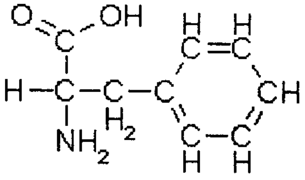
Valine

- Sulphur containing : methionine (Met, M).

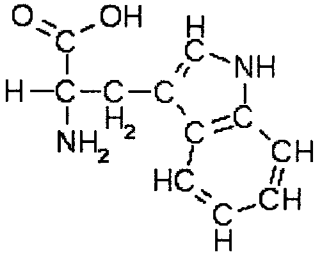


Methionine

- Aromatic : phenylalanine (Phe, F), tryptophan (Trp, W).



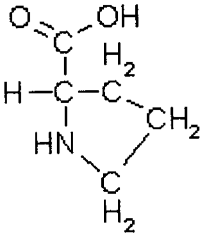
Phenylalanine



Tryptophan

-

- Cyclic imine : proline (Pro, P).

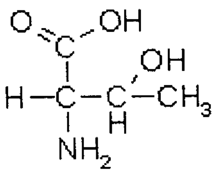


Proline

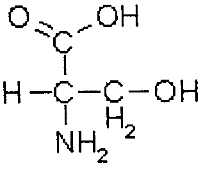
Proline has an unusual imine ring structure (a secondary amine), where the terminal amine group is actually incorporated into the side chain. This causes changes to the secondary structure of a protein. Hydrophobic residues are often found in membrane bound proteins, and the aromatic ones contribute to protein absorbance at 280 nm, which is an important method of protein quantification. Alanine is the 'don't care' amino acid, often appearing where nothing interesting is happening!

The polar amino acids are those that are not charged at physiological pH, but which are nevertheless quite polar due to their alcohol or amide groups.

- Alcohols : threonine (Thr, T), serine (Ser, S).

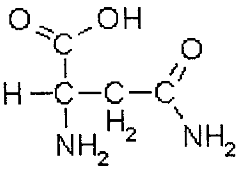


Threonine

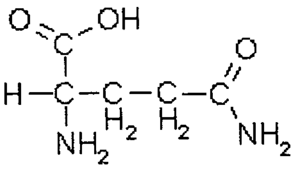


Serine

- Amides : asparagine (Asn, N), glutamine (Gln, Q).



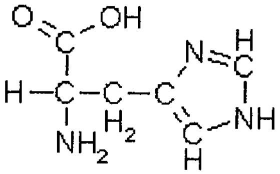
Asparagine



Glutamine

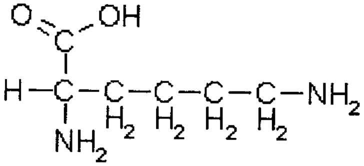
The basic amino acids are those that can accept protons. The pK_a refers to the dissociation of the proton from a positively charged (protonated) amine group.

- Imidazole : histidine (His, H, pK$_a$ of protonated form of the group -C=NH$^+$- = 6.04).



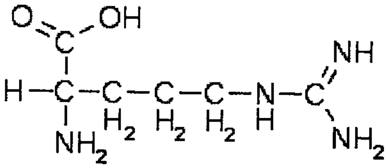
Histidine

- Amine: lysine (Lys, K, pK$_a$ of protonated form of terminal amine group NH$_3^+$ = 10.54).



Lysine

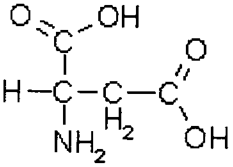
- Guanidinium : arginine (Arg, R, pK$_a$ of protonated form of terminal group -C=NH$_2^+$ = 12.48).



Arginine

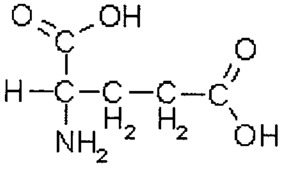
The acidic amino acids are carboxylic acids, plus an thiol (cysteine) and a phenol (tyrosine) with dissociable S-H or O-H groups:

- Aspartic acid (Asp, D, pK$_a$ of COOH group = 3.90).



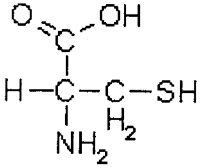
Aspartic acid

- Glutamic acid (Glu, E, pK_a of COOH group = 4.07).



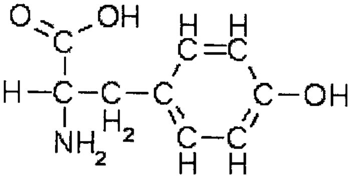
Glutamic acid

- Cysteine (Cys, C, pK_a of SH group = 8.37).



Cysteine

- Phenol: tyrosine (Tyr, Y, pK_a of OH group = 10.46).



Tyrosine

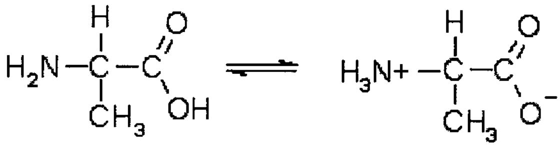
The sodium salt of glutamic acid is the flavour enhancer MSG.

Cysteine is capable of forming a dimer: Cys-SH + Cys-SH = Cys-S-S-Cys. These disulphide bridges (confusingly known as cystine), are responsible for a lot of protein tertiary structures.

The charged (acidic/basic) and polar amino acids are often involved in catalysis, forming covalent products with substrates. The terminal COOH and NH_2 groups on an amino acid (and on larger peptides and proteins) may also be charged, as have their own pKa's.

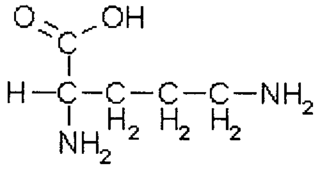
- NH_3^+ has pK_a of *c.* 9.5
- COOH has pK_a of *c.* 2.5

At physiological pH, amino acids and proteins are usually charged at both ends, a *zwitterion.*

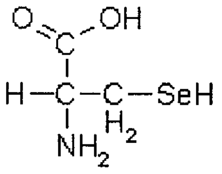


Alanine zwitterion.

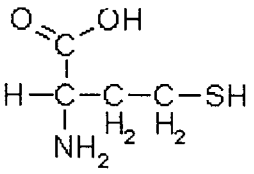
In addition to the amino acids found in proteins, the cell also contains a number of other amino acids that are not normally found in peptides. This includes ornithine, an important intermediate in the urea cycle, selenocysteine, a very rare component of some proteins and homocysteine, an important intermediate in sulphur metabolism.



Ornithine

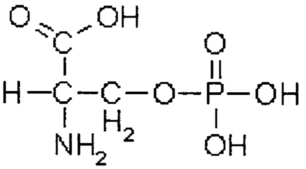


Selenocysteine

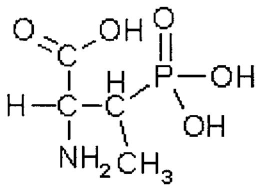


Homocysteine

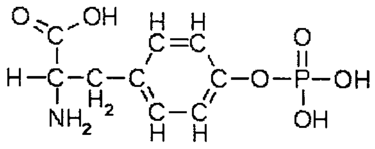
In addition to this, many amino acids in proteins are modified after translation to incorporate phosphate groups, extra hydrogen, *etc.* These modified amino acid residues include the phosphorylated alcohol amino acids (Ser, Thr, Tyr), which are phosphorylated by kinase enzymes. This is a common strategy for protein regulation. The histone proteins, which regulate DNA packaging in the nucleus are themselves regulated by methylation of their arginine and lysine residues, and the characteristic triple helical structure of collagen is caused by its possession of many glycine and hydroxyproline (a post-translational modification of proline) residues.



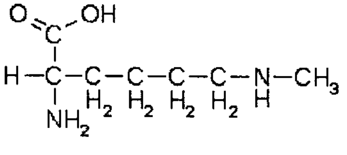
O-phosphoserine



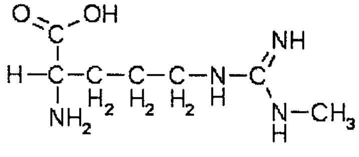
O-phosphothreonine



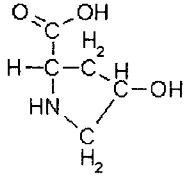
O-phosphotyrosine



***N*-Methyllysine**

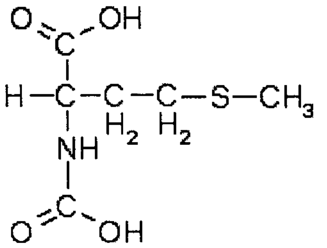


***N*-Methylarginine**

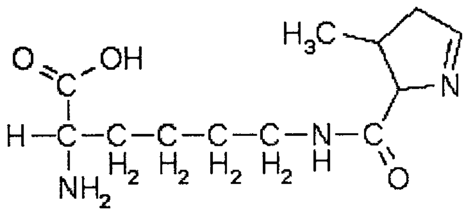


Hydroxyproline

One final interesting amino acid modification is that found in the methanogenic Archaea. These 'bacteria' interpret the amber stop codon as the amino acid methylpyrrolysine, making this the 22nd genetically encoded amino acid (the 21st being the *N*-formyl methionine that eukaryotes use to start translating all their proteins).



N-formyl methionine



Methylpyrrolysine

All amino acids share two chemically functional groups, the carboxyl group and the amino group. Thus, they will share the chemical reactions of these groups familiar from organic chemistry. Many of these reactions are exploited in the laboratory manipulation of amino acids, peptides, and proteins. Note that these reactions are also common to the side chains of asp, glu (-COOH), and lys (-NH_2). Another side-chain with important chemistry is cys (-SH). Biologically the most important reactions are those required for protein formation, particularly the peptide bond.

Classification of Amino Acids

The protein amino acids are classified according to the chemical nature of their R groups as aliphatic, aromatic, heterocyclic and sulphur containing amino acids. More meaningful classification of amino acids is based on the polarity of the R groups. The polarity of the R groups varies widely from totally non-polar to highly polar. The 20 amino acids are classified into four main classes.

- **Amino acids with non-polar or hydrophobic, aliphatic R groups**

This group of amino acids includes glycine, alanine, valine, leucine. Isoleucine and proline. The hydrocarbon R groups are non-polar and hydrophobic. The side chains of alanine, valine, leucine and isoleucine are important in promoting hydrophobic interactions within protein structures. On the other hand, the imino group of proline is held in a rigid conformation and reduces the structural flexibility of the protein.

- **Amino acids with non-polar aromatic R groups.**

This group includes phenylalanine, tyrosine and tryptophan. All these amino acids participate in hydrophobic interactions, which is stronger than aliphatic R groups because of stacking one another. Tyrosine and tryptophan are more polar than phenylalanine due to the presence of hydroxyl group in tyrosine and nitrogen in the indole ring of tryptophan. The absorption of ultraviolet (UV) light at 280 nm by tyrosine, tryptophan and to a lesser extent by phenylalanine is responsible for the characteristic strong absorbance of light by proteins. This property is exploited in the characterization and quantification of proteins.

- **Amino acids with polar, uncharged R groups.**

This group of amino acids includes serine, threonine, cysteine, methionine, asparagine and glutamine. The hydroxyl group of serine and threonine, the sulphur atom of cysteine and methionine and the amide group of asparagine and glutamine, contribute to the polarity. The R groups of these amino acids are more hydrophilic than the non-polar amino acids.

- **Amino acids with charged R groups.**

Acidic : The two amino acids with acidic R groups are aspartic and glutamic acids. These amino acids have a net negative charge at pH 7.0.

Basic : This group includes lysine, arginine and histidine. The R groups have a net positive charge at pH 7.0. The lysine has a second ε-amino group; arginine has a positively charged guanidino group; and histidine has an imidazole group.

Essential Amino Acids

Most of the prokaryotic and many eukaryotic organisms (plants) are capable of synthesizing all the amino acids present in the protein. But higher animals including man possess this ability only for certain amino acids. The other amino acids, which are needed for normal functioning of the body but cannot be synthesized from metabolic intermediates, are called essential amino acids. These must be obtained from the diet and a deficiency in any one of the amino acids prevents growth and may even cause death. Methionine, Threonine, Tryptophan, Valine, Isoleucine, Leucine, Phenylalanine and Lysine are the essential amino acids, however, Histidine and Arginine are considered semi essential amino acids as it can be partly synthesized by the body.

Properties of Amino Acids

Physical

- Amino acids are white crystalline substances. Most of them are soluble in water and insoluble in non-polar organic solvents (e.g., chloroform and ether).

- Aliphatic and aromatic amino acids particularly those having several carbon atoms have limited solubility in water but readily soluble in polar organic solvents.
- They have high melting points varying from 200-300^{o}C or even more.
- They are tasteless, sweet or bitter. Some are having good flavour.
- Amphoteric nature of amino acids as they contain both acidic (COOH) and basic (NH2) groups. The amino acids possessing both positive and negative charges are called zwitterions.
- Isomerism - All amino acids except proline, found in protein are α-amino acids because NH2 group is attached to the α-carbon atom, which is next to the COOH group. Examination of the structure of amino acids reveals that except glycine, all other amino acids possess asymmetric carbon atom at the position.

Chemical Properties

- Reaction with formaldehyde (Formal titration): An amino acid solution is treated with excess of neutralized formaldehyde solution, the amino group combines with formaldehyde forming dimethylol amino acid which is an amino acid formaldehyde complex Hence the amino group is protected and the proton released is titrated against alkali. This method is used to find out the amount of total free amino acids in plant samples.

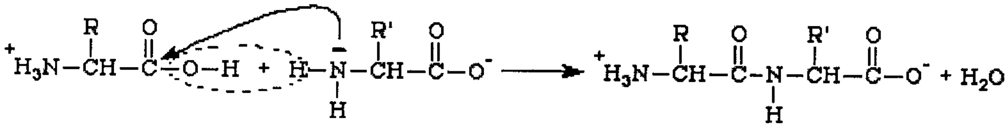
- Reaction with nitrous acid: Nitrous acid reacts with the amino group of amino acids to form the corresponding hydroxyacids and liberate nitrogen gas.
- Reaction with ninhydrin: Ninhydrin is a strong oxidizing agent. When a solution of amino acid is boiled with ninhydrin, the amino acid is oxidatively deaminated to produce ammonia and a ketoacid. The keto acid is decarboxylated to produce an aldehyde with one carbon atom less than the parent amino acid. The net reaction is that ninhydrin oxidatively deaminates and decarboxylates α-amino acids to CO2, NH3 and an aldehyde. The reduced ninhydrin then reacts with the liberated ammonia and another molecule of intact ninhydrin to produce a purple coloured compound known as Ruhemann's purple.

- This ninhydrin reaction is employed in the quantitative determination of amino acids. Proteins and peptides that have free amino group(s) (in the side chain) will also react and give colour with ninhydrin.
- Decarboxylation: The carboxyl group of amino acids is decarboxylated to yield the corresponding amines. Thus, the vasoconstrictor agent, histamine

is produced from histidine. Histamine stimulates the flow of gastric juice into the stomach and the dilation and constriction of specific blood vessels. Excess reaction to histamine causes the symptoms of asthma and various allergic reactions.

Peptides

Amino acids are linked together by formation of covalent bonds. The covalent bond is formed between the α-carboxyl group of one amino acid and the α-amino group of the next amino acid. The bond so formed between the carboxyl and the amino groups, after elimination of a water molecule is called as a peptide bond and the compound formed is a peptide.



$$^{+}H_3N-\overset{R}{\overset{|}{C}}H-\overset{O}{\overset{\|}{C}}-O-H + H-\underset{H}{\underset{|}{N}}-\overset{R'}{\overset{|}{C}}H-\overset{O}{\overset{\|}{C}}-O^{-} \longrightarrow {}^{+}H_3N-\overset{R}{\overset{|}{C}}H-\overset{O}{\overset{\|}{C}}-\underset{H}{\underset{|}{N}}-\overset{R'}{\overset{|}{C}}H-\overset{O}{\overset{\|}{C}}-O^{-} + H_2O$$

- The peptide bond is formed with the elimination of water, giving a planar bond between the carboxyl carbon and the amino nitrogen.
- Linear peptides will have free amino- and carboxy- terminal groups. Thus they will exhibit titration curves similar to a free amino acid, but with the pK_a values shifted closer to simple acid and amine values (there will be no charge stabilization).
- By convention the amino terminal residue is written on the left progressing to the carboxyl terminal residue on the right: $^{+}H_3N$-aa-aa-aa-aa-CO_2^{-}.
- Can determine the composition of a peptide by acid hydrolysis and amino acid analysis.
- Can sequence proteins by specific enzyme and chemical hydrolysis to give peptides which can then be run through sequenators (up to about 100 aa's).
- Amino acid sequences have been used to help determine relatedness of organisms.

Classification of Protein : Proteins are classified based on their

(A) Solubility and composition.
(B) Function.
(C) Size & Shape.

(A) Classification of Proteins Based on Solubility and Composition

Proteins are again divided into three main groups as simple, conjugated and derived proteins.

(I) Simple Proteins

Simple proteins yield on hydrolysis, only amino acids. These proteins are further classified based on their solubility in different solvents as well as their heat coagulability.

Albumins

Albumins are readily soluble in water, dilute acids and alkalies and coagulated by heat. Seed proteins contain albumin in lesser quantities. Albumins may be precipitated out from solution using high salt concentration, a process called 'salting out'. They are deficient in glycine. Example - Serum albumin and Ovalbumin (egg white).

Globulins

Globulins are insoluble or sparingly soluble in water, but their solubility is greatly increased by the addition of neutral salts such as sodium chloride. These proteins are coagulated by heat. They are deficient in methionine. Example - Serum globulin, Fibrinogen, Myosin of muscle and Globulins of pulses.

Prolamins

Prolamins are insoluble in water but soluble in 70-80% aqueous alcohol. Upon hydrolysis they yield much proline and amide nitrogen. They are deficient in lysine. Example - Gliadin of wheat and Zein of corn.

Glutelins

Glutelins are insoluble in water and absolute alcohol but soluble in dilute alkalies and acids. They are plant proteins. Example - Glutenin of wheat.

Histones

Histones are small and stable basic proteins and contain fairly large amounts of basic amino acid, histidine. They are soluble in water, but insoluble in ammonium hydroxide. They are not readily coagulated by heat. They occur in globin of haemoglobin and nucleoproteins.

Protamines

Protamines are the simplest of the proteins. They are soluble in water and are not coagulated by heat. They are basic in nature due to the presence of large quantities of arginine. Protamines are found in association with nucleic acid in the sperm cells of certain fish. Tyrosine and tryptophan are usually absent in protamines.

Albuminoids

These are characterized by great stability and insolubility in water and salt solutions. These are called albuminoids because they are essentially similar to albumin and globulins. They are highly resistant to proteolytic enzymes. They are fibrous in nature and form most of the supporting structures of animals. They occur as chief constituent of exoskeleton structure such as hair, horn and nails.

(II) Conjugated or Compound Proteins

These are simple proteins combined with some non-protein substances known as prosthetic groups. The nature of the non-protein or prosthetic groups is the basis for the sub classification of conjugated proteins.

Nucleoproteins

Nucleoproteins are simple basic proteins (protamines or histones) in salt combination with nucleic acids as the prosthetic group. They are the important constituents of nuclei and chromatin.

Mucoproteins

These proteins are composed of simple proteins in combination with carbohydrates like mucopolysaccharides, which include hyaluronic acid and chondroitin sulphates. On hydrolysis, mucopolysaccharides yield more than 4% of amino-sugars, hexosamine and uronic acid e.g., ovomucoid from egg white. Soluble mucoproteins are neither readily denatured by heat nor easily precipitated by common protein precipitants like trichloroacetic acid or picric acid. The term glycoproteins is restricted to those proteins that contain small amounts of carbohydrate usually less than 4% hexosamine.

Chromoproteins

These are proteins containing coloured prosthetic groups e.g., haemoglobin, flavoprotein and cytochrome.

Lipoproteins

These are proteins conjugated with lipids such as neutral fat, phospholipids and cholesterol.

Metalloproteins

These are metal-binding proteins. A β-globulin, termed transferrin is capable of combining with iron, copper and zinc. This protein constitutes approximately 3% of the total plasma protein. Another example is ceruloplasmin, which contains copper.

Phosphoproteins

These are proteins containing phosphoric acid. Phosphoric acid is linked to the hydroxyl group of certain amino acids like serine in the protein, Example- casein of milk.

(III) Derived Proteins

These are proteins derived by partial to complete hydrolysis from the simple or conjugated proteins by the action of acids, alkalies or enzymes. They include two types of derivatives, primary-derived proteins and secondary-derived proteins.

Primary-derived Proteins

These protein derivatives are formed by processes causing only slight changes in the protein molecule and its properties. There is little or no hydrolytic cleavage of peptide bonds.

Proteans

Proteans are insoluble products formed by the action of water, dilute acids and enzymes. These are particularly formed from globulins but are insoluble in dilute salt solutions. Example - myosan from myosin, fibrin from fibrinogen.

Metaproteins

These are formed by the action of acids and alkalies upon protein. They are insoluble in neutral solvents.

Coagulated Proteins

Coagulated proteins are insoluble products formed by the action of heat or alcohol on natural proteins. Example - cooked meat and cooked albumin.

Secondary-derived Proteins

These proteins are formed in the progressive hydrolytic cleavage of the peptide bonds of protein molecule. They are roughly grouped into proteoses, peptones and peptides according to average molecular weight. Proteoses are hydrolytic products of proteins, which are soluble in water and are not coagulated by heat. Peptones are hydrolytic products, which have simpler structure than proteoses. They are soluble in water and are not coagulated by heat. Peptides are composed of relatively few amino acids. They are water-soluble and not coagulated by heat. The complete hydrolytic decomposition of the natural protein molecule into amino acids generally progresses through successive stages as follows:

Protein ------→ Protean -------→ Metaprotein ------→
Proteoses ------→ Peptones -------→ Peptides ------→ amino acids

(B) Classification of Proteins Based on Function

Proteins are classified based on their functions as -

(I) Catalytic Proteins - Enzymes

The most striking characteristic feature of these proteins is their ability to function within the living cells as biocatalysts. These biocatalysts are called as enzymes. Enzymes represent the largest class. Nearly 2000 different kinds of enzymes are known, each catalyzing a different kind of reaction. They enhance the reaction rates a million fold.

(II) Regulatory Proteins - Hormones

These are polypeptides and small proteins found in relatively lower concentrations in animal kingdom but play highly important regulatory role in maintaining order in complex metabolic reactions.Example- growth hormone, insulin etc.

(III) Protective Proteins - Antibodies

Some proteins have protective defense function. These proteins combine with foreign protein and other substances and fight against certain diseases.Example - immunoglobulin. These proteins are produced in the spleen and lymphatic cells in response to foreign substances called antigen. The newly formed protein is called antibody which specifically combines with the antigen which triggered its synthesis thereby prevents the development of diseases. Fibrin present in the blood is also a protective protein.

(IV) Storage Proteins

A major class of proteins which has the function of storing amino acids as nutrients and as building blocks for the growing tissues. Storage proteins are source of essential amino acids, which cannot be synthesized by human beings. The major storage protein in pulses is globulins and prolamins in cereals. But in rice the major storage protein is glutelins. Albumin of egg and casein of milk are also storage proteins.

(V) Transport Proteins

Some proteins are capable of binding and transporting specific types of molecules through blood. Haemoglobin is a conjugated protein composed of colourless basic protein, the globin and ferroprotoporphyrin or haem. It has the capacity to bind with oxygen and transport through blood to various tissues. Myoglobin, a related protein, transports oxygen in muscle. Lipids bind to serum proteins, principally, albumin and transported as lipoproteins in the blood.

(VI) Toxic Proteins

Some of the proteins are toxic in nature. Ricin present in castor bean is extremely toxic to higher animals in very small amounts. Enzyme inhibitors such as trypsin inhibitor bind to digestive enzyme and prevent the availability of the protein. Lectin, a toxic protein present commonly in legumes, agglutinates red blood cells. A bacterial toxin causes cholera, which is a protein. Snake venom is protein in nature.

(VII) Structural Proteins

Some proteins serve as structural materials or as important components of extra cellular fluid. Examples of structural proteins are myosin of muscles, keratin of skin and hair and collagen of connective tissue. Carbohydrates, fats, minerals and other cellular components are organized around such structural proteins that form the molecular framework of living material.

(VIII) Contractile Proteins

Proteins like actin and myosin function as essential elements in contractile system of skeletal muscle.

(IX) Secretary Proteins

Fibroin is a protein secreted by spiders and silkworms to form webs and cocoons.

(X) Exotic Proteins

Antarctic fishes live in -1.9^{0}C waters, well below the temperature at which their blood is expected to freeze. These fishes are prevented from freezing by antifreeze glycoproteins present in their body.

(C) Classification of Proteins Based on Size and Shape

Based on size and shape, the proteins are also subdivided into globular and fibrous proteins. Globular proteins are mostly water-soluble and fragile in nature. Example- enzymes, hormones and antibodies. Fibrous proteins are tough and water-insoluble. They are used to build a variety of materials that support and protect specific tissues. Example- Skin, Hair, Fingernails and Keratin.

Conformation of Proteins

Conformation of a protein refers to the three-dimensional structure in its native state. There are many different possible conformations for a molecule as large as a protein. Four types of structural organization can be distinguished in proteins: (Fig. 5.1)

- Primary
- Secondary
- Tertiary
- Quaternary

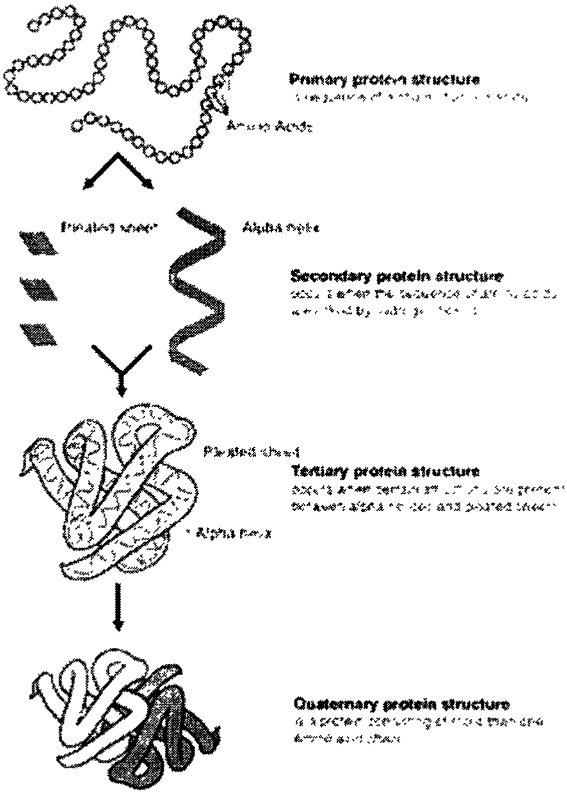

Primary protein structure
Amino Acids
Pleated sheet
Alpha helix
Secondary protein structure
Pleated sheet
Tertiary protein structure
Alpha helix
Quaternary protein structure

Fig. 5.1 : Structural Organization of Proteins

Primary structure

The linear order or sequence of peptide bonded amino acid residues, beginning at the N-terminus. (Characteristic bond type: covalent).

Primary structure of protein refers to the number of amino acids and the order in which they are covalently linked together. It also refers to the location of disulfide bridges, if there are any, in a polypeptide chain (Fig 5.2).

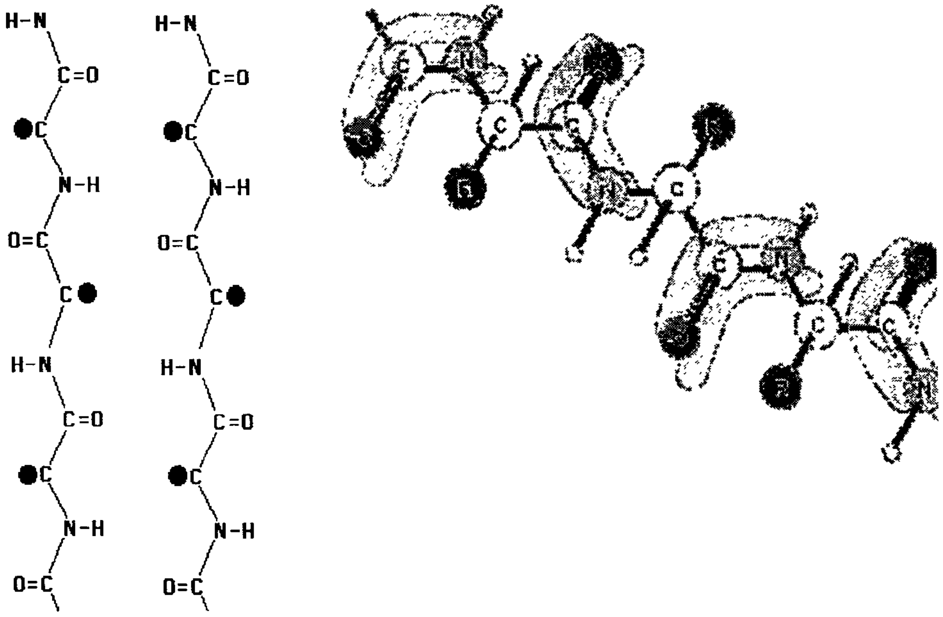


Fig. 5.2 : Primary Structure of Proteins

Frederick Sanger in 1953 determined the complete amino acid sequence of insulin for the first time. The important steps involved in determining the primary structure of protein are -

- Determination of number of (chemically different) polypeptide chains or subunits in the protein.
- Separation of polypeptide chains if more than one are present in a protein.
- Determination of the amino acid sequence of the subunits.
- Elucidation of the position of the disulphide bonds, if any, between and within the subunits.

Edman's reagent is also used to determine the amino acid sequence of a polypeptide chain from the N-terminal by subjecting the polypeptide to repeated cycles of Edman degradation. After every cycle, the newly liberated phenylthiohydantoin (PTH) amino acid was identified. The sequence of peptides containing 30-40 amino acids can be determined using a sequencer by adopting the Edman's degradation method.

C-terminal identification: C-terminal amino acid can be determined by methods similar to those used for the N-terminal acid. Hydrazine is used to find out the C-terminal amino acid.

Amino Acid Sequencing of Polypeptides

The amino acid sequence in polypeptides with 30-40 amino acids can be determined by Edman reaction. For polypeptides containing more than 40 amino acids, both enzymatic and chemical methods are employed to break polypeptide chains into smaller peptides. The enzyme, trypsin hydrolyses the peptide bond on the carboxyl side of the basic amino acid residues of lysine or arginine. The chemical reagent, cyanogen bromide cleaves peptide bond on the carboxyl side of methionine residues. The hydrolyzed peptides are separated and the amino acid sequence is determined by Edman reaction. The hydrolysis of the original polypeptide by two different methods separately gives overlapping regions, from which the sequence is derived.

Secondary Structure

The steric relations of residues nearby in the primary structure which give rise to local regularities of conformation. These structures are maintained by hydrogen bonds between peptide bond carbonyl oxygens and amide hydrogens. The major secondary structural elements are the helix and the beta strand. (Characteristic bond type: hydrogen.)

Secondary structure refers to the steric relationship of amino acids that are close to one another in the linear sequence. The folding of a linear polypeptide chain occurs to form a specific coiled structure. Such coiling or folding is maintained by hydrogen bonds and hydrogen bond is the only bond responsible for secondary structure. X-ray studies of several polypeptides by Linus Pauling and Robert Corey revealed that the peptide group has a rigid, planar structure which is a consequence of resonance interactions that give the peptide bond a 40% double bond character. Peptide groups mostly assume the trans-conformation in which successive C2 atoms are on opposite sides of peptide bond joining them. The cis configuration creates steric interference. If a polypeptide chain is twisted by the same amount each of its C atoms, it assumes a helical conformation (Fig 5.3).

Helix Structure

The α-helix is the most stable arrangement of polypeptides. The helix structure of proteins is stabilized by intramolecular hydrogen bonding.

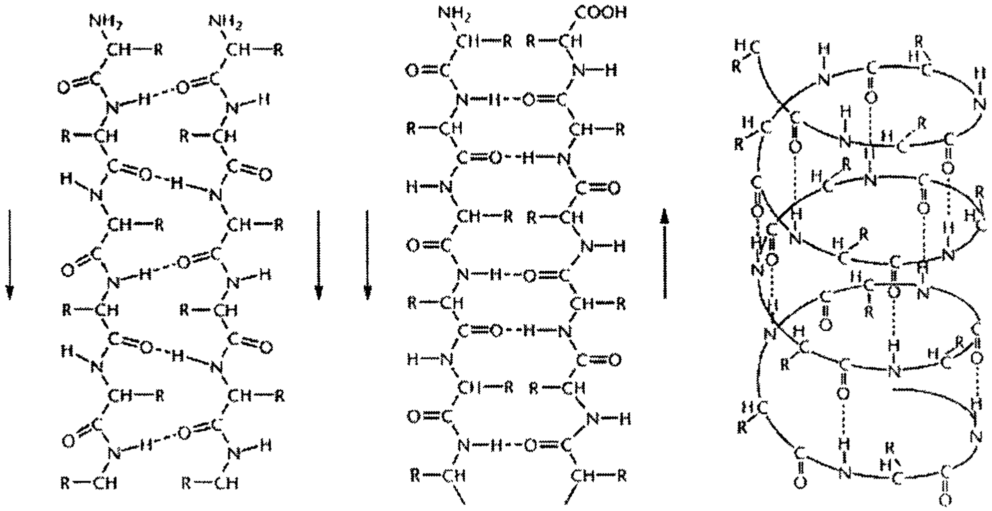
In this structure, hydrogen bonds are formed between the C=O group of one peptide bond and the N-H group of another after 3 amino acid units. The polypeptide chain constituted by L-amino acids form a right-handed helix, whereas the polypeptide chains made up of D-amino acids form a left-handed helix. In the α-helical conformation, all the side chains lie outside the helix whereas C, N, O and H of the peptide bond lie in the same plane.

Certain amino acids tend to disrupt the α-helix. Among these are proline (the N-atoms is part of the rigid ring and no rotation of the N-C bond can occur) and amino acid with charged or bulk R groups that either electrostatically or physically interferes with helix formation.

The β-pleated Sheet Structure

Pauling and Corey also proposed a second ordered structure, the β-pleated sheet, for polypeptide. This structure is a result of intermolecular hydrogen bonding between the polypeptide chains to form a sheet like arrangement (Fig. 5.3).

There are two ways in which proteins chains can form the pleated sheet structure. One is with the chains running in the same direction i.e. the -COOH or NH2 ends of the polypeptide chains lying all at the top or all at the bottom of the sheet. This is called parallel pleated-sheet structure. In another type, known as antiparallel β-pleated sheet structure, the polypeptide chains alternate in such a way that the -COOH end of the one polypeptide is next to the -NH2 end of the other i.e., polypeptide chains run in opposite directions.



Parallel β pleated sheet

Antiparallel β pleated sheet

Right-handed α helix

Fig. 5.3 : Secondary Structure of Protein

The Random Coil

Regions of proteins that are not identifiably organized as helices or pleated sheets are said to be present in random coil conformation. Considerable portion of the protein may be present in this conformation. The term 'random' is unfortunate which imply less biological significance than more highly repeating regions. But in terms of biological function, the regions of random coil are of equal importance to those of helix and pleated sheet.

Tertiary Structure

The steric relations of residues distant in the primary sequence; the overall folding pattern of a single covalently linked molecule. (Characteristic bond type: hydrophobic; others: hydrogen, ion-pair, van der Waals, disulphide.)

Hydrogen Bonds

Hydrogen bonds are formed between the side chain (R group) of amino acids having a hydrogen donor group and an acceptor group.

Salt-linkages **(electrostatic forces; ionic bonds)**

Salt linkages are due to the interaction between amino groups of basic amino acids and the carboxyl group of acidic amino acids present in the R group.

Disulphide Bonds **(S-S linkages)**

The S-S linkages are formed by the oxidation of sulfhydryl (-SH) group of two cysteine side chains.

Hydrophobic Bonds

Hydrophobic bonds are formed as a result of interaction between non-polar side chains.

Dipole-dipole Interaction

This interaction occurs between polar unionized side chains.

The folding of a polypeptide chain due to different covalent and non-covalent interactions (Fig. 5.4).

Out of the above bonds, the disulphide bond (covalent bond) is the strongest and cannot be affected by solvent, pH, temperature and salts whereas the above conditions. The disulphide bond can be split and reformed by oxidation/reduction respectively. The tertiary structure gains special importance in the case of enzymes.

Domain

Independent folding regions within a protein. The group/pattern of secondary structures forming a Domain's tertiary structure is called a **Fold**. (Characteristic bond type: hydrophobic; others: hydrogen, ion-pair, van der Waals.)

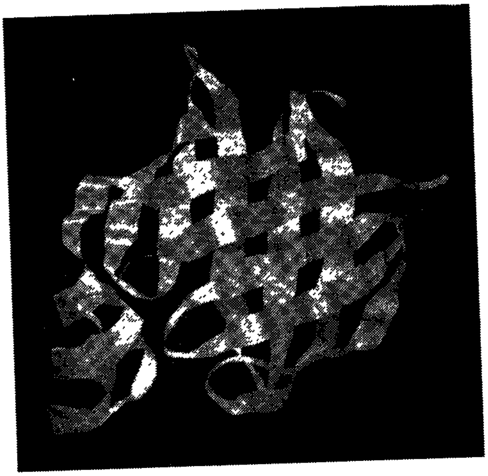


Fig. 5.4 : Teritary Structure of Proteins

A long peptide strand of a protein will often fold into multiple, compact semi-independent folded regions or domains. Each domain having a characteristic spherical geometry with a hydrophobic core and polar surface very much like the tertiary structure of a whole globular protein. The domains of a multidomain protein are often interconnected by a segment of polypeptide chain lacking regular secondary structure. In enzymes with more than one substrate or allosteric effector sites the different binding sites are often located in different domains. In multifunctional proteins, the different domains perform different tasks.

Quaternary Structure

The association of two or more independent proteins via non-covalent forces to give a multimeric protein. The individual peptide units of this protein are referred to as subunits, and they may be identical or different from one another. (Characteristic bond type: hydrophobic; others: hydrogen, ion-pair, van der Waals.)

Proteins that have more than one subunit or polypeptide chains will exhibit quaternary structure (Fig. 5.5). Quaternary structure refers to a functional protein aggregate (organization) formed by interpolypeptide linkage of subunits or polypeptide chains. These subunits are held together by noncovalent surface interaction between the polar side chains. Proteins formed like above are termed oligomers and the individual polypeptide chains are variously termed protomers, monomers or subunits. The most common oligomeric proteins contain two or four protomers and are termed dimers or tetramers. respectively.

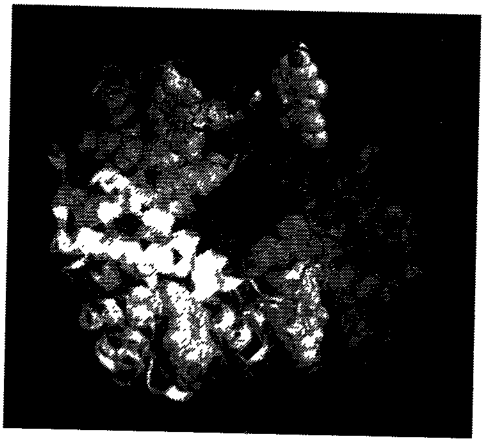


Fig. 5.5 : Quaternary Structure of Protein

Myoglobin has no quaternary structure since, it is composed of a single polypeptide chain. Hemoglobin molecule, which consists of four separate polypeptide chains, exhibits quaternary structure.

Quaternary structure may influence the activity of enzymes. Some enzymes are active only in their quaternary state and become inactive when split into smaller units. Other enzymes are inactive in the quaternary state and are activated only when they are dissociated to form monomeric state.

Rationale for Quaternary : There are a variety of advantages to large structures:

- Increasing the size of a protein allows better "fits" for catalysis and binding - many weak bonds are needed to maintain specific structures.
- Can bring sequential active sites of metabolic pathways into close proximity.
- However, large peptides have some problems:
    - The process of folding slows tremendously with increasing size, thus folding individual subunits, and assembling these subunits can greatly enhance folding efficiency.
    - Get about 1 error / 10^3 aa residues due to the precision of the translation of messenger RNA to protein. Thus, need to keep residue number down.
- Interacting subunits provide mechanisms for regulation.

Physical and Chemical Properties of Proteins

Physical Properties

- Pure proteins are generally tasteless, though the predominant taste of protein hydrolysates is bitter.
- Pure proteins are odourless.
- Because of the large size of the molecules, proteins exhibit many properties that are colloidal in nature.
- Proteins, like amino acids, are amphoteric and contain both acidic and basic groups.
- They possess electrically charged groups and hence migrate in an electric field.
- Many proteins are labile and readily modified by alterations in pH, UV radiation, heat and by many organic solvents.
- The absorption spectrum of protein is maximum at 280 nm due to the presence of tyrosine and tryptophan, which are the strongest chromophores in that region. Hence the absorbance of the protein at this wavelength is adapted for its determination.

Denaturation of protein - The comparatively weak forces responsible for maintaining secondary, tertiary and quaternary structure of proteins are readily disrupted with resulting loss of biological activity. This disruption of native structure is termed denaturation.

Physical and chemical factors are involved in the denaturation of protein :

- Heat and UV radiation supply kinetic energy to protein molecules causing their atoms to vibrate rapidly, thus disrupting the relatively weak hydrogen bonds and salt linkages. This results in denaturation of protein leading to coagulation. Enzymes easily digest denatured or coagulated proteins.
- Organic solvents such as ethyl alcohol and acetone are capable of forming intermolecular hydrogen bonds with protein disrupting the intramolecular hydrogen bonding. This causes precipitation of protein.
- Acidic and basic reagents cause changes in pH, which alter the charges present on the side chain of protein disrupting the salt linkages.
- Salts of heavy metal ions (Hg2+, Pb2+) form very strong bonds with carboxylate anions of aspartate and glutamate thus disturbing the salt linkages. This property makes some of the heavy metal salts suitable for use as antiseptics.

Renaturation - Renaturation refers to the attainment of an original, regular three-dimensional functional protein after its denaturation.

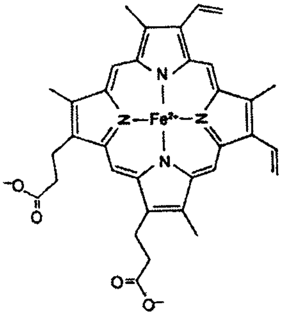
When active pancreatic ribonuclease A is treated with 8M urea or β-mercaptoethanol, it is converted to an inactive, denatured molecule. When urea or mercaptoethanol is removed, it attains its native (active) conformation.

Chemical Properties

- Colour Reactions of Proteins - The colour reactions of proteins are of importance in the qualitative detection and quantitative estimation of proteins and their constituent amino acids. Biuret test is extensively used as a test to detect proteins in biological materials.
- Biuret Reaction - A compound, which is having more than one peptide bond when treated with Biuret reagent, produces a violet colour. This is due to the formation of coordination complex between four nitrogen atoms of two polypeptide chains and one copper atom.
- Xanthoproteic Reaction - Addition of concentrated nitric acid to protein produces yellow colour on heating, the colour changes to orange when the solution is made alkaline. The yellow stains upon the skin caused by nitric acid are the result of this xanthoproteic reaction. This is due to the nitration of the phenyl rings of aromatic amino acids.
- Hopkins-Cole Reaction - Indole ring of tryptophan reacts with glacial acetic acid in the presence of concentrated sulphuric acid and forms a purple coloured product. Glacial acetic acid reacts with concentrated sulphuric acid and forms glyoxalic acid, which in turn reacts with indole ring of tryptophan in the presence of sulphuric acid forming a purple coloured product.

Myoglobin and Haemoglobin

Myoglobin (Mb) : Myoglobin is a 153 residue globular protein in the globin family. Eight helices form its single domain (myoglobin fold) tertiary structure; about 80% helix (high for globular proteins). Interior almost exclusively hydrophobic residues, with water excluded from interior. Surface has mix of hydrophobic and hydrophilic residues, with ionizable groups on surface.



Myoglobin functions to store and facilitate the diffusion of oxygen in muscle. Oxygen binds to a heme {Fe (II)-protoporphyrin IX} prosthetic grp. Four of irons six ligands are to heme nitrogens, with a fifth to a histidine nitrogen. The final ligand bond goes to oxygen. *Breathing motions* are necessary to allow the exchange of oxygen, since the heme is in a closed pocket.

- Single chain, high α-helical content, globular.
- Heme Prosthetic Group.
- Co-factor: iron, Fe2+ (ferrous), the binding of O2 keeps Fe2+ from oxidizing to Fe3+.
- Six coordination sites.
- Four sites are occupied by nitrogen (relatively the same plane).
- Fifth site by nitrogen from the proximal histidine residue .
- Sixth site by oxygen or carbon monoxide (nitrogen from distal histidine). (Fig. 5.6)

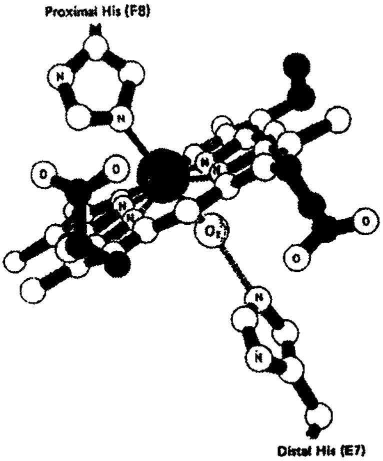


Fig. 5.6 : Structure of Myoglobin

Carbon monoxide is a toxic by-product of metabolism; heme actually has a higher affinity for CO then O2.

Mb and Hb have developed a mechanism (unknown) to discriminate against binding by CO, still, CO binds better than O2 and a measurable amount of Mb and Hb in the body exist bound to CO.

Table 5.1 : Myoglobin - Normal O_2 Pressures:

Location	**Approximate pO2 (mmHg)**	**Comments**
Air	150	Normal atmospheric pressure at sea level
Lung Capillaries	100	Where direct gas exchange occurs
Venous Blood	40	Where gas exchange to tissues occurs
Muscle	20	Under normal activity
O2-Deprived Muscle	>5	Under conditions of strenuous exercise

Myoglobin binds oxygen when the pO2 is high and releases oxygen at very low pO2. (Table 5.1, Fig. 5.2)

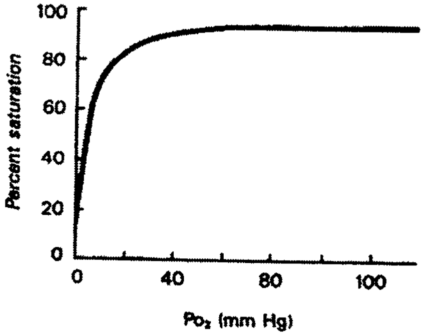


Fig. 5.2 : Oxygen Affinity Curve for Myoglobin

- When all Mb molecules have O2 bound-100%
- When no Mb molecules have O2 bound-0%

Looking at Curve :

1. High affinity for O2, at most physiological conditions O2 remains bound to Mb.
2. Only when O2 falls very low, exercise etc., Mb releases O2 to other tissues.
3. The role of Mb is to store O2 for release during crisis involving O2 deprivation i.e., seals and whales contain abundant Mb (deep dives).

Haemoglobin (Hb): Haemoglobin is an --beta-beta oligomeric protein: its quaternary structure consists of a tetramer of myoglobin like subunits. The two types of chain are slightly shorter than myoglobin chains (= 141 amino acid residues, beta = 146 amino acid residues). There are extensive contacts between an and a beta subunit to give a dimer. The dimers have additional contacts to give the tetramer. Oxygen binding results in a change of conformation in Hb. The change of conformation affects the binding of oxygen {oxygen binding is reduced in the "blue" form due to steric hindrance between the oxygen and the haeme}.

- Structure very similar to Mb.
- Exception: tetramer, two types of subunits.
- Two α and two β (α2β2, referred to as hba-normal hemoglobin).
- Cooperativity/Cooperative Binding.
- When an O2 molecule binds to the first site a conformational change occurs in the tetrameric structure which affects binding to the other subunits.
- Oxygen binding curve is a characteristic sigmoidal ("S") shape.

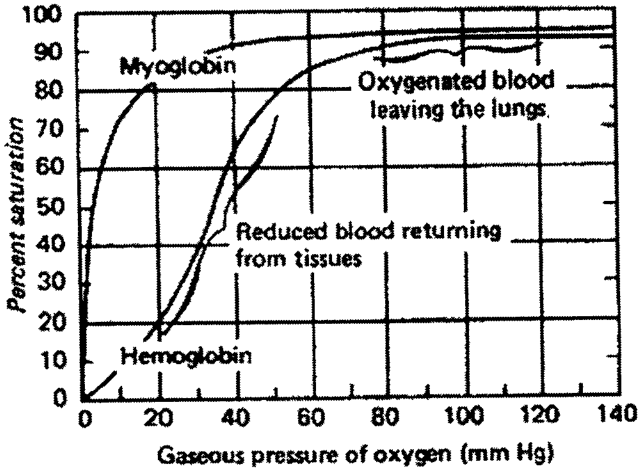


Fig. 5.7 : Comparison of Oxygen Affinity Curve for Hemoglobin vs. Myoglobin

- Hb takes up O2 in the lungs and releases it to interior tissues.
- At pO2of 100 mmHg, Hb is saturated.
- At pO2 of 20 mmHg, Hb loses most of its O2.
- Even at 40 mmHg Hb is loosing some of it's O2.

Note:

- The amount of Hb in the blood is so high that it contributes to the buffering capacity of blood.
- Bicarbonate system is still the major blood buffer, however
- Hb does have the ability to accept or donate protons through its His residues (36 His residues per tetramer, so this cannot be discounted....).

Allosterism and Regulation

Allosteric ("other site") enzyme or binding proteins are proteins with multiple **interacting** sites. Allosteric proteins can exhibit one or both of two types of allosterism:

- Homotropic: this is where the sites are identical, and each sites is allosteric to the others. This is like the cooperative interactions seen in oxygen binding by haemoglobin - four (essentially) identical oxygen binding sites interacting with each other allosterically.
- Heterotropic: this is where binding at one kind of site affects the binding at a second kind of site. This occurs in the regulation of oxygen binding in haemoglobin by BPG (BisPhosphoGlycerate = DPG in older literature). BPG affects the binding of oxygen by all of the oxygen sites. [Hb overheads].

Look at cooperativity/regulation curves for enzymes/binding proteins. Get two families of regulators:

- Positive (+) effectors or activators. These shift the binding constant so the binding takes place at lower concentrations, or increases the rate of an enzyme at a given concentration. Positive (+) effectors **decrease cooperativity**.
- Negative (-) effectors : Decrease binding, so concentration must be increased to give the same level of activity. Negative (-) effectors **increase cooperativity**.

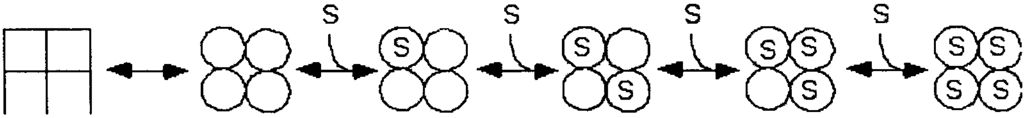
So how to explain the cooperative behaviour of allosteric proteins? Need to explain both kinds of effects.

- Homotropic allosterism as occurs with O_2 binding in Hb.
- Heterotropic regulation of O_2 binding, as occurs with BPG in Hb.

Two Important Models: Symmetry Model of Allosterism and Sequential Model of Allosterism.

Symmetry Model of Allosterism

- Allosteric protein have an oligomer of protein subunits that are symmetrically related;
- Protomers can exist in two conformations (designated T[ense]: low affinity for substrate, & R[elaxed]: high affinity for substrate) that are in equilibrium whether or not ligand is bound;
- Ligand can bind to protomers in either conformation, but conformational change alters affinity for the ligand;
- The molecular symmetry of the protein is conserved during conformational change: the protomers must therefore change in a concerted manner.



- It can also be added binding of effectors to this model: positive effectors bind to R (circles) and shift equilibrium to right, negative effectors bind to T (squares) and shift equilibrium to left.

Sequential Model of Allosterism.

In this model the subunits are each influenced by binding to other subunits, but change is step-wise rather than concerted.

QUIZ

Identify the letter of the choice that best completes the statement or Answers the question.

1. There are ___ commonly occurring amino acids which makes proteins.
   A. 4 B. 5 C. 40 D. 20

   Answer: D

2. At neutral pH (pH = 7):
   A. the carboxyl group exists as -COOH and the amino group exists as -NH3+
   B. the carboxyl group exists as -COO- and the amino group exists as -NH3+
   C. the carboxyl group exists as -COO- and the amino group exists as -NH2-
   D. the carboxyl group exists as -COOH and the amino group exists as -NH2-

   Answer: B

3. At neutral pH (pH = 7):
A. acidic amino acids have a net positive charge
B. basic amino acids have a net positive charge
C. acidic amino acids have no charge
D. basic amino acids have no charge

Answer: B

4. Which of the following is not a nonpolar amino acid?
A. isoleucine B. valine
C. methionine D. arginine

Answer: D

5. Which of the following amino acids contain(s) a sulfur atom?
A. leucine serine B. methionine, cysteine
C. histidine D. arginine

Answer: B

6. The hormone epinephrine (also known as adrenaline) is derived from which amino acid?
A. phenylalanine B. glycine
C. tyrosine D. histidine

Answer: C

7. Which of the following amino acid(s) is (are) neutral and polar at pH = 7?
A. tyrosine B. serine
C. asparagine D. all of the above

Answer: D

8. Amino acids can be readily detected and quantified by reaction with:
A. zwitterion
B. hydroxyproline
C. 5,5'-Dithiobis (2-nitrobenzoic acid)
D. ninhydrin (triketohydrindene hydrate)

Answer: D

9. Which of the following statements is false regarding enantiomers?
A. Enantiomeric molecules display optical activity.
B. Enantiomers are nonsuperimposable mirror image isomers.
C. Enantiomeric molecules are able to rotate the plane of polarization of plane-polarized light.
D. If the direction of the polarized light is clockwise, it is referred to as levorotatory behavior.

Answer: D

10. Which of the following amino acids has (have) two chiral centers?
A. threonine B. proline
C. isoleucine D. A and C

Answer: D

11. Which of the following amino acids exhibit(s) significant ultraviolet absorption above 250 nm?

A. alanine B. tyrosine
C. methonine D. glycine

Answer: B

12. Which of the following statements is true about the spectroscopic properties of amino acids?

A. None of the amino acids absorbs light in the visible region of the electromagnetic spectrum.
B. None of the amino acids absorbs ultraviolet radiation.
C. None of the amino acids absorbs infrared region.
D. None of the amino acids absorbs sunlight.

Answer: A

13. Which of the following principles is (are) false?

A. The chemical shift of amino acid protons depends on their particular chemical environment.
B. The chemical shift of amino acid protons depends on the state of ionization of the amino acid.
C. The change in electron density during a titration is not transmitted throughout the carbon chain in the aliphatic amino acids and aliphatic protons of aromatic amino acids.
D. The magnitude of the coupling constants between protons on adjacent carbons depends in some cases on the ionization state of the amino acid.

Answer: C

14. Which of the following best describes the order of elution of a mixture of asparagine, lysine, and serine (from first to last) from a cation exchange column?

A. A, L, S B. G, S, K C. N, L, A D. N, S, K

Answer: D

15. Which of the following is (are) not a cation exchanger?

A. Basic polystyrene resin (Dowex-1)
B. Diethylaminoethyl (DEAE)
C. Acidic polystyrene resin (Dowex 50)
D. A and B

Answer: D

16. Most proteins absorb light at a wavelength of 280 nm. Which of the following amino acids is primarily responsible for this absorption?

A. glycine B. histidine
C. proline D. tryptophan

Answer: D

17. Which of the following amino acids lacks an asymmetric carbon atom?
    A. serine B. glycine
    C. histidine D. glutamine

Answer: B

18. Isomers that differ at only one of the asymmetric centers are called:
    A. diastereomers B. dextrorotatory
    C. levorotatory D. prochiral

Answer: A

19. The partial double bond character of the peptide bond:
    A. restricts rotation around the C-N bond
    B. allows free rotation around the C-N bon
    C. restricts rotation around the Ca-C bond
    D. restricts rotation around the N-Ca bond

Answer: A

20. The peptide linkage between amino acids has partial double bond character. Because of this
    A. There is free rotation around the peptide bond.
    B. The carbonyl and amino groups always reside on the same side of the bond.
    C. The cis conformation is favored over the trans conformation.
    D. There is restricted rotation about the peptide bond.

Answer: D

21. The type of bond holding the amino acid units together in a dipeptide molecule is called:
    A. a glycosidic bond
    B. an ester bond
    C. a peptide bond
    D. a protein bond

Answer: C

22. Proteins are broken down into their amino acids by:
    A. a hydrolysis reaction
    B. a decomposition reaction
    C. a polymerisation reaction
    D. an addition reaction

Answer: A

23. All amino acids have the following pair of functional groups:
    A. amine and an ester
    B. carboxylic acid and an amine
    C. amine and alcohol
    D. alcohol and carboxylic acid

Answer: B

25. Two amino acid molecules combine to form a dipeptide molecule. The reaction that occurs is:
   A. an oxidation reaction
   B. a condensation reaction
   C. a hydrolysis reaction
   D. an esterification reaction

Answer: B

26. Essential amino acids can be:
   A. made from non-essential amino acids
   B. obtained by eating the correct food
   C. made from amines
   D. made in the body

Answer: B

27. When complex biomolecules such as proteins are unfolded and disordered from their biologically active, or native, forms, the process is referred to as:
   A. culmination B. de-energization
   C. hydrolysis D. denaturation

Answer: D

28. Phosphoric acid, H_3PO_4:
   A. is a polyprotic acid.
   B. requires two equivalents of OH- to neutralize it.
   C. is a strong acid.
   D. has a pKa of 2.38.

Answer: A

29. The arrangement of the two alpha and two beta polypeptide chains in hemoglobin is an example of the _______ structure of a protein.
   A. primary B. secondary
   C. tertiary D. quaternary

Answer: D

30. Which of the following noncovalent interactions are NOT important in protein structure?
   A. hydrophobic interactions B. ionic bonds
   C. electrostatic bonds D. van der Waals forces

Answer: B

31. Each hydrogen bond contributes ~ ____ kJ/mol in stabilization energy to the protein structure.
   A. 1.2 B. 12 C. 120 D. 1,200 e. 40,000

Answer: B

32. Which of the following statements about hydrophobic bonds is false?
    - A. The forming of hydrophobic bonds maximizes the interaction of nonpolar residues with water.
    - B. They form because nonpolar side chains of amino acids and other nonpolar solutes prefer to cluster in a nonpolar environment.
    - C. Hydrophobic bonds can also be referred to as hydrophobic interactions.
    - D. They form because nonpolar side chains prefer not to intercalate in a polar solvent such as water.

Answer: A

33. Which of the following statements about electrostatic interactions is false?
    - A. They arise either as electrostatic attractions between opposite charges or repulsions between like charges.
    - B. Electrostatic interactions between charged groups on a protein surface are often complicated by the presence of salts.
    - C. They are important for protein stability.
    - D. Most proteins do not have amino acids that participate in electrostatic interactions.

Answer: D

34. Which of the following statements about Van der Waals interactions is false?
    - A. They can arise due to instantaneous dipole-induced dipole interactions.
    - B. They consist of both attractive forces and repulsive forces.
    - C. Individual van der Waals interactions are weak.
    - D. Because they are so weak, they do not contribute much to protein stability.

Answer: D

35. The resonance stabilization energy of the planar structure of the peptide bond is approximately:
    - A. 0.88 kJ/mol B. 8.8 kJ/mol
    - C. 88 kJ/mol D. 800 kJ/mol

Answer: C

36. One turn of an a-helix represents ___ amino acid residues.
    - A. 2.6 B. 3 C. 3.6 D. 4

Answer: C

37. Which of the following statements is (are) true about an a-helix?
    - A. The side chains extend outward from the core structure of the helix.
    - B. All of the H-bonds lie parallel to the helix axis.
    - C. All of the carbonyl groups point in one direction along the helix axis.
    - D. All of the above are true.

Answer: D

38. In hemoglobin, which is capable of reversibly binding oxygen, the iron atom at the center of the heme group is in:
    A. the +3 ferric state B. the +3 ferrous state
    C. the +2 ferric state D. the +2 ferrous state

Answer: D

39. Which of the following statements about beta-pleated sheets (b-sheets) is false?
    A. Adjacent chains of amino acids that form b-sheets always run in the same direction (N --> C or C --> N).
    B. They arise due to cooperative formation of hydrogen bonds.
    C. The hydrogen bonds in this structure are essentially interstrand rather than intrastrand.
    D. The side chains in the pleated b-sheet are oriented perpendicular to the plane of the sheet.

Answer: A

40. Which of the following statements about fibrous proteins is false?
    A. They contain polypeptide chains organized approximately parallel along a single axis.
    B. The organization of the polypeptide chains produces long fibers or large sheets.
    C. They tend to be mechanically weak.
    D. They resist solubilization in water and dilute salt solutions.

Answer: C

41. Collagen fibers have a high content of which amino acids?
    A. histidine, tyrosine, and proline
    B. proline, hydroxyproline, and tyrosine
    C. alanine, phenalanine, and isoleucine
    D. glycine, proline, and hydroxyproline

Answer: D

42. Which of the following statements about globular proteins is true?
    A. They are not as abundant in nature as fibrous proteins.
    B. They do not contain substantial amounts of b-sheets.
    C. It is common in globular protein structures for one face of an a-helix to be exposed to the water solvent, with the other face toward the hydrophobic interior of the protein.
    D. Collagen is an example of a globular protein.

Answer: C

43. Which of the following motions occur(s) in proteins?
    A. Atomic vibrations
    B. Collective motions

C. Conformational changes
D. All

Answer: D

44. The term used to refer to how individual polypeptide chains of a protein having two or more chains are arranged in relation to each other is:
A. primary structure B. secondary structure
C. tertiary structure D. Quaternary structure

Answer: D

45. Which of the following sets of amino acid residues would you most likely find on the exterior surface of a globular protein at physiological pH?
A. Phe, Asn, Glu, Trp B. Ala, Phe, Asp, Glu
C. Thr, Met, Glu, Arg D. Glu, Asp, Arg, Lys

Answer: D

46. The basic structural unit of collagen, tropocollagen, has the following structure:
A. a triple-stranded extended helix B. a beta-pleated sheet
C. a right-handed alpha-helix D. a left-handed alpha-helix

Answer: A

47. The affinity of hemoglobin for oxygen is increased by:
A. a higher H+ concentration in the surroundings
B. an increase in the organic phosphate level in red cells
C. an increase in the CO2 concentration in the surroundings
D. an increase in the oxygen concentration in the surroundings

Answer: D

48. 2,3-bisphosphoglycerate lowers the affinity for oxygen of hemoglobin by:
A. binding preferentially to deoxyhemoglobin
B. binding preferentially to oxy-hemoglobin
C. stabilizing oxy-hemoglobin by cross-linking the beta chains
D. binding to the ferrous form of heme groups

Answer: A

49. A characteristic of sickle cell hemoglobin, Hb S, is that it:
A. will not bind oxygen
B. has abnormal heme groups
C. contains only alpha globin chains
D. tends to form aggregates when deoxygenated

Answer: D

50. In hemoglobin, which is capable of reversibly binding oxygen, the iron atom at the center of the heme group is in:
A. the +3 ferric state B. the +3 ferrous state
C. the +2 ferric state D. the +2 ferrous state

Answer: D

51. Motor proteins convert chemical energy in the form of ________ into the mechanical energy of motion.
    A. ATP B. a-tubulin C. cyclic AMP D. glucose

    Answer: A

52. a-tubulin and b-tubulin dimers polymerize to form ____________, a rudimentary component of the euraryote cytoskeleton.
    A. actin B. cilia C. microtubules D. axons

    Answer: C

53. The following observation is a key element in support of the sliding filament model of muscle contraction:
    A. The thick and thin filaments' lengths are constant during contraction.
    B. The myosin thick filament expands, while the actin thin filament contracts to achieve maximal force of contraction.
    C. The constant length of thick and thin filaments provides a rigid structure for titin molecules to perform the contraction.
    D. The thick and thin filaments change lengths in proportional amounts.

    Answer: A

54. The power stroke of the myosin heads during muscle contraction immediately follows __________.
    A. ATP hydrolysis B. ATP association
    C. ADP release D. ADP binding

    Answer: C

55. ATP binding to myosin causes ___________ of myosin with actin.
    A. association B. polymerization
    C. dimerization D. dissociation

    Answer: D

56. Which word best describes proteins that are enzymes and immunoglobins:
    A. pleated B. linear
    C. coiled D. globular

    Answer: D

57. Proteins that have a quaternary structure have:
    A. a protein and a nucleic acid bonded together
    B. a protein and a lipid bonded together
    C. two or more proteins bonded together
    D. a protein and a carbohydrate bonded together

    Answer: C

58. The sequence of amino acids in a protein is called its:
    A. tertiary structure
    B. quaternary structure
    C. primary structure

D. secondary structure

Answer: C

59. Which of the following types of bond is *not* present within or between protein molecules:
    - A. ionic bonds
    - B. covalent bonds between oxygen atoms
    - C. hydrogen bonds
    - D. covalent bonds between sulphur atoms

Answer: B

60. __________ are proteins with multiple interacting sites.
    - A. Protective proteins
    - B. Storage proteins
    - C. Allosteric binding proteins
    - D. None

Answer: C

Complete each sentence or statement.

1. The ________ is the end of a polypeptide that has a free carboxylate group.

Answer: C-terminus

2. ________ is the asymmetry or "handedness" of a molecule such that it cannot be superimposed on its mirror image.

Answer: Chirality

3. A segment of a polymer in which the backbone adopts a regularly repeating conformation is called a/an ________.

Answer: regular secondary structure

4. R state and T state are the two conformations of a/an ________.

Answer: allosteric protein

5. A/An ________ is the formation of a covalent bond between two molecules during which the elements of water are lost.

Answer: condensation reaction

6. An accumulation of certain types of insoluble protein aggregates in tissues is a/an ________..

Answer: amyloid deposit

7. A loop is a segment of a/an ________, usually found on the protein surface, that joins two elements of secondary structure.

Answer: polypeptide

8. ________ is an enzyme that catalyzes the hydrolysis of peptide bonds.

Answer: Protease or Proteinase or Peptidase

9. ________ is a procedure for the stepwise removal and identification of the N-terminal residues of a polypeptide.

Answer: Edman degradation

10. Irregular secondary structure is a segment of a polymer in which each residue has a different ________ conformation.

Answer: backbone

11. The record of the radiation scattered from an object, for example in X-ray crystallography, is called a/an ________.

Answer: diffraction pattern

12. A/An ________ is a protein that binds to unfolded or misfolded proteins in order to promote their normal folding.

Answer: molecular chaperone

13. In a heteropolymer, the ________ are not all identical.

Answer: residues

14. ________ is the local spatial arrangement of a polymer's backbone atoms without regard to the conformations of its substituent side chains.

Answer: Secondary structure

15. A stretch of polypeptide residues that fold into a globular unit with a hydrophobic core is called a/an ________.

Answer: domain

16. An ion pair is an electrostatic interaction between two ionic groups of ________ charge.

Answer: opposite

17. ________ is the refolding of a denatured macromolecule so as to regain its native conformation.

Answer: Renaturation

18. An allosteric protein is a protein in which the binding of a/an ________ at one site affects their binding at other sites.

Answer: ligand

19. A group's ________ is determined by the chemical properties of the group's immediate neighbors.

Answer: microenvironment

20. The fully folded conformation of a macromolecule is its ________.

Answer: native structure or native conformation

21. In ________ binding, the binding of a ligand at one site on a macromolecule affects the affinity of other sites for the same ligand.

Answer: cooperative

22. A/An ________ is an essential organic group that is permanently associated with an enzyme.

Answer: prosthetic group

23. A change of an amino acid residue in a protein to one with similar properties is a/an ________.

Answer: conservative substitution

24. A/An ________ residue in a protein is the same in all evolutionarily related proteins.

Answer: invariant

25. ________ is hemoglobin that does not contain bound oxygen or is not in the oxygen-binding conformation.

Answer: Deoxyhaemoglobin

26. The decrease in O2 binding affinity of hemoglobin in response to a decrease in pH is called the ________.

Answer: Bohr effect

27. ________ is an inflammatory disease usually caused by impaired uric acid excretion and characterized by painful deposition of uric acid in the joints.

Answer: Gout

28. A triple helix is the right-handed helical structure formed by three left-handed helical polypeptide chains in ________.

Answer: collagen

29. The addition of monomeric units to one end of a polymer and their removal from the opposite end such that the length of the polymer remains unchanged is called ________.

Answer: treadmilling

30. ________ is a property of a motor protein or other enzyme that undergoes many reaction cycles before dissociating from its track or substrate.

Answer: Processivity

31. The ________ is the network of intracellular fibers that gives a cell its shape and structural rigidity.

Answer: cytoskeleton

32. The polymerized form of the protein actin is called ________.

Answer: F-actin

33. Microfilament-stiffened cell processes, called ________, occur on the surface of cells in the inner ear and are deflected in response to sound waves.

Answer: stereocilia

34. A/An ________ is one of the 13 linear polymers of tubulin subunits that forms a microtubule.

Answer: protofilament

35. An arrangement of polypeptide chains in which two helices wind around each other is called a/an ________.

Answer: coiled coil

36. ________ is a genetic disease characterized by elastic skin and joint hyperextensibility and caused by mutations in genes for collagen or collagen-processing proteins.

Answer: Ehlers-Danlos syndrome

37. ________ is the spread of tumor cells to other sites in the body.

Answer: Metastasis

38. ________ is a disease caused by mutations in collagen genes and characterized by bone fragility and deformation.

Answer: Osteogenesis imperfecta

39. A muscle cell structural element that consists primarily of an actin filament is a/an ________.

Answer: thin filament

40. ________ is the splitting of the cell into two following mitosis.

Answer: Cytokinesis

41. Collagen is an extracellular fibrous protein found primarily in ________ and connective tissue.

Answer: bone

42. A/An ________ protein binds to the exposed end of a polymeric molecule preventing further polymerization or depolymerization.

Answer: capping

43. ________ is the monomeric form of the protein actin.

Answer: G-actin

44. ________ is a genetic disease characterized by profound deafness and retinitis pigmentosa that leads to blindness and in some cases is caused by a defective myosin protein.

Answer: Usher syndrome

45. A muscle cell structural element that is composed of several hundred myosin molecules is a/an ________.

Answer: thick filament

46. ________ is a technique for determining molecular structure in which the electron beam of an electron microscope is used to elicit diffraction from a two-dimensional crystal of the molecules of interest.

Answer: Electron crystallography

“This Page is Intentionally Left Blank”

Chapter 6

Enzymes and Kinetics

Enzymes are the heart of Biochemistry.

- Protein based catalysts.
- Enormously effective catalysts: typically enhance rates by 10^6 to 10^{12} fold.
- Operate under mild conditions: 0 - 100 °C (or perhaps even 300+ °C for some bacteria in deep ocean), 20 - 40 °C for most organisms; and low pressures (atmospheric).
- Very Specific: Generally catalyze reaction for a very restricted group of molecules, sometimes for a single naturally occurring molecule of a single chirality.

Enzymes generally have a cleft for active site, generally <5%of surface: look like pac man. Need large structure to maintain shape etc., with many weak bonds.

One of the unique characteristics of a living cell is its ability to permit complex reactions to proceed rapidly at the temperature of the surrounding environment. The principal agents which participate in the remarkable transformations in the cell belong to a group of proteins named enzymes. In the absence of enzymes in the cell, these reactions would proceed too slowly. Enzymes are proteins specialized to catalyse biological reactions with the following characteristics.

Characteristics of Enzymes

Enzymes being proteins exhibit all properties of proteins. They have their specific isoelectric points at which they are least soluble. Like proteins, they can be denatured by changes in pH and temperature. The enzyme-catalysed reactions occur below 100^0C, at atmospheric pressure and nearby neutral pH.

Enzymes undergo physical changes during the reaction but revert to their original form at the end of the reaction. Enzymes exhibit enormous catalytic power. The rates of enzymatically catalysed reactions are 106 - 1012 times greater than those of the corresponding uncatalysed reactions and several times greater than those of the corresponding chemically catalysed reactions. For example the carbonic anhydrase enzyme catalyses the conversion of carbondioxide to carbonic acid.

$$CO_2 + H_2O \rightarrow H_2CO_3$$

In this reaction, each enzyme molecule can hydrate 105 molecules of carbondioxide per second.

Enzyme activity is regulated in a variety of ways, ranging from controls over the amount of enzyme protein synthesised by the cell or modulation of activity through reversible interaction with metabolic inhibitors and activators or through isoenzymes.

Specificity of the Enzymes

One of the characteristic feature which distinguishes enzymes from catalysts is their specificity. Enzymes are specific in the reaction catalysed and in their choice of substrates. It usually catalyses a single chemical reaction or a set of closely related reactions. Three kinds of specificities are observed.

Absolute Specificity

When enzymes catalyse only one particular reaction they are said to exhibit absolute specificity. e.g., Urease acts only on urea.

Group Specificity

Enzymes acting on a group of substances that possess a particular type of linkage common to that group of substances are said to exhibit group specificity. Amylase hydrolyses the group of substances like starch, dextrin and glycogen, which have the same type of glycosidic linkages (α 1,4).

Optical Specificity

Almost all enzymes show a high degree of optical specificity. Thus, there are certain enzymes which catalyse the hydrolysis of same group of substances possessing same optical activity i.e., D-amino acid oxidase acts on D-amino acid and L-amino acid oxidase acts on L-amino acid. Maltase catalyses the hydrolysis of α-but not β- glycosides.

Classification of Enzymes

The International Union of Biochemistry (IUB) established a commission on enzyme nomenclature to adopt a systematic classification and nomenclature of all the existing and yet to be discovered enzymes. This system is based on the substrate and reaction specificity. Although, this International Union of Biochemistry system is complex, it is precise, descriptive and informative.

IUB system classifies enzymes into six major classes (should be written in specific order only).

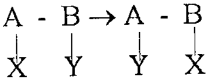
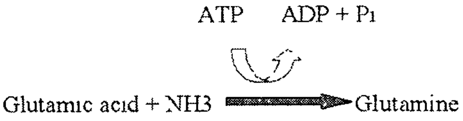
1. Oxidoreductases

2. Transferases
3. Hydrolases
4. Lyases
5. Isomerases
6. Ligases

Again each class is divided into subclasses according to the type of reaction catalysed. Each enzyme is assigned a recommended name usually a short one for everyday use, a systematic name which identify the reaction it catalyses and a classification number which is used where accurate and unambiguous identification of an enzyme is required (Table 6.1).

Table 6.1 : Classification and Functions of Enzymes

Main Classifi-cation Number	Major Classes and Sub-classes	Type of Reaction Catalysed
1.	**Oxidoreductases** Dehydogenases Oxidases Reductases Peroxidases Catalases Hydroxylases	Transfer of electrons usually in the form of hydrogen atoms or hydride ions A reduced + B oxidised → A oxidsed + B reduced CH3-CH2-OH + NAD^+---→CH3-CHO + NADH + H^+ (reduced) (oxidised) (oxidised) (reduced)
2.	**Transferases** Kinases Acyltransferases Transaldolases Methyl transferases	Transfer of functional groups form one molecule to another. Example: Phosphorylation of glucose by hexokinase $A - B + C \rightarrow A + B - C$
3.	**Hydrolases** Esterases Glycosidases Peptidses Phosphatases Thiolases Phospholipases Amidases	Cleavage of bonds by hydrolysis $A\text{-}B + H_2O \text{--------}\rightarrow A\text{-}H + B\text{-}OH$
4.	**Lyases** Decarboxylases Aldolases Hydratases Dehydratases	Removal of groups by a mechanism other than hydrolysis leaving a double bond in one of the product. $\underset{X}{A} - \underset{Y}{B} \rightarrow A - B + X\text{-}Y$

5.	**Isomerases** Racemases Epimerases Isomerases Mutases	Transfer of groups within a molecule to yield isomeric forms  $\underset{X}{A} - \underset{Y}{B} \rightarrow \underset{Y}{A} - \underset{X}{B}$
6.	**Ligases** Synthetases Carboxylases	Formation of new bonds(C-C, C-S, C-O C-N etc.) by condensation coupled with hydrolysis of high energy molecules like ATP). $A - B + C \rightarrow A + B - C$  $\text{Glutamic acid} + NH_3 \xrightarrow{ATP \quad ADP + P_i} \text{Glutamine}$ Enzyme· Glutamine synthetase

Comparision of Apoenzymes, Coenzymes and Cofactors

A large number of enzymes require an additional non-protein component to carry out its catalytic functions. Generally these non-protein components are called as cofactors. The cofactors may be either one or more inorganic ions such as Fe2+, Mg2+, Mn2+ and Zn2+ or a complex organic molecules called coenzymes. Some enzymes require both coenzyme and one or more metal ions for their activity. A coenzyme or metal ion that is covalently bound to the enzyme protein is called prosthetic group. A complete, catalytically active enzyme together with its coenzyme and/or metal ions is called holoenzyme. The protein part of such an enzyme is called apoenzyme or apoprotein. Coenzymes function as transient carriers of specific functional groups

Cofactors

Metals are required as cofactors in approximately two thirds of all enzymes. Metalloenzymes contain a definite quantity of functional metal ion that is retained throughout. Metal-activated enzymes bind metals less tightly but require added metals. The distinction between metalloenzymes and metal activated enzymes thus rests on the affinity of a particular enzyme for its metal ion. The mechanisms whereby metal ions perform their function appear to be similar both in metallo enzymes and metal activated enzymes. Metals participate through their ability to act as Lewis acids and through chelate formation. Example of a metal functioning as a Lewis acid is the zinc in carbonic anhydrase. The metal can also promote catalysis by binding substrate at the site of bond cleavage. In carboxypeptidase, the carbonyl oxygen is chelated to the zinc.

Enzymes Requiring Metal Ions as Cofactors

Enzymes	Cofactors
Cytochrome oxidase	Fe^{2+} and Cu^{2+}
Peroxidase, catalase	Fe^{2+} or Fe^{3+}
Carbonic anhydrase	Zn^{2+}
Alcohol dehydrogenase	Zn^{2+}
Hexokinase	Mg^{2+}
Pyruvate kinase	Mg^{2+}
Glucose 6-phosphatase	Mg^{2+}
Pyruvate kinase	K^{+}
Nitrogenase, nitrate reductase	Mo
Glutathione peroxidase	Se

Mechanism of Enzyme Action

A chemical reaction such as $A \rightarrow P$ takes place because a certain fraction of the substrate possesses enough energy to attain an activated condition called the transition state. This transition state is at the top of the energy barrier separating the reactants and products. The rate of a given chemical reaction is proportional to the concentration of this transition state species. The energy of activation is the amount of energy required to bring all the molecules in 1 mole of a substance at a given temperature to the transition state. Enzymes combine transiently with the substrate to produce a transition state intermediate having a lower energy of activation than the uncatalysed reaction. Thus, they accelerate chemical reactions by lowering the energy of activation.

Example

$$H_2O_2 \xrightarrow[\text{Catalase}]{} H_2O + (O)$$

Reaction Condition	Activation Energy (KCal mol-1)
Uncatalysed	18
Catalysed by colloidal Pt	13
Catalysed by catalase	7

It is generally believed that the catalytic reactions occur in atleast two steps:

Step 1: A molecule of enzyme(E) and a molecule of substrate(S) collide and react to form an intermediate called the enzyme-substrate complex (ES).

Step 2: The decomposition of ES complex to give product(s) and the active enzyme

$$[S] + [E] \longrightarrow [ES] \longrightarrow P + [E]$$

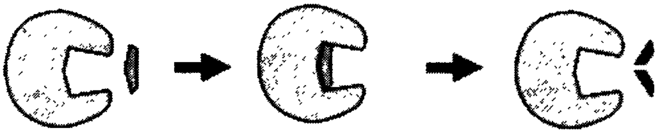
The formation of an ES complex affords a lower activation energy.

Active Site

The functional groups that are essential for the formation of ES complex occur at a specific location on the surface of the enzyme molecule. This section of enzyme where substrate binding and transformation of substrate to product occurs is called as active site. Many attempts have been made to implicate specific amino acid residues (side chain or R groups) as being part of the active site of various enzymes. Some of the amino acids occurring at the active site of enzymes are hydroxyl group of serine, sulfhydryl group of cysteine, imidazole group of histidine and carboxyl group of aspartic acid. Two theories were proposed to explain the mechanism of enzyme action.

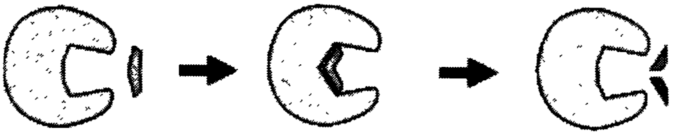
Fischer's Lock and Key Theory (Rigid Template Model)

According to this theory proposed by Emil Fischer during 1890s, the active site possesses a unique conformation which is complementary to the structure of the substrate thus enabling the two molecules to fit together in much the same way as a key fits into a lock. An unfortunate feature of this model is the implied rigidity of the catalytic site.



Koshland's Induced-fit Theory

Koshland had advocated a theory to account for the specificity of enzymes. He postulated that the essential functional groups on the active site of the free enzyme are not in their optimal positions for promoting catalysis. When the substrate molecule is bound by the enzyme the catalytic groups assume a favourable geometrical position to form the transition state. The enzyme molecule is unstable in this active conformation and tends to revert to its free form in the absence of substrate. In the induced fit model, the substrate induces a conformational change in the enzyme which aligns the amino acid residues or other groups for substrate binding, catalysis or both.



Enzymes Kinetics and Inhibitors

Factors Affecting Enzymatic Reaction

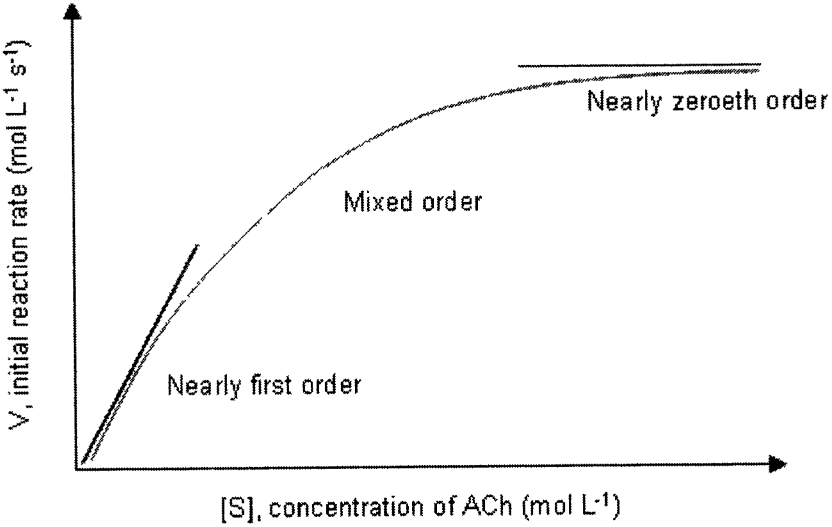
The factors that mainly influence any enzyme-catalysed reaction are :

1. Substrate concentration
2. Enzyme concentration
3. Temperature
4. pH
5. Inhibitors

Other factors such as state of enzyme (oxidation), time and activators also affect enzyme-catalysed reaction to certain extent.

Substrate Concentration

Keeping the factors such as pH, temperature and enzyme concentration at optimum levels, if the substrate concentration is increased, the velocity of the reaction recorded a rectangular hyperbola. At very low substrate concentration the initial reaction velocity (v) is nearly proportional to the substrate concentration (first order kinetics). However, if the substrate concentration is increased the rate of increase slows down (mixed order kinetics). With a further increase in the substrate concentration the reaction rate approaches a constant (zero order-reaction where velocity is independent of substrate concentration).



At initial point, eventhough the substrate molecules are present in excess than enzyme on molar basis, not all the enzyme molecules present combine with the substrate. Hence, increasing the substrate concentration will increase the amount of enzyme associated with substrate as ES and thus reaction will depend on [S]. At Vmax, all the enzyme molecules are saturated with substrate molecules so that further increase in [S] cannot result in increased reaction rate. Michaelis-Menten derived an equation to explain this type of behaviour (Fig. 6.1).

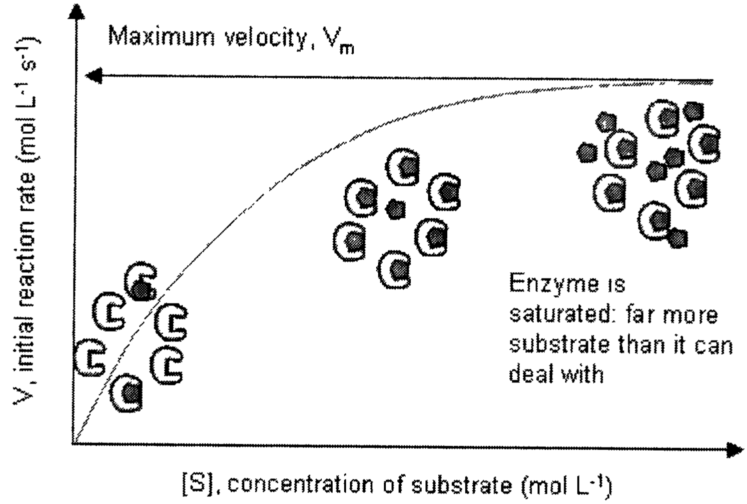


Fig. 6.1 : Michaelis-Menten Curve and Enzyme-Substrate Relationship

Let's look at a mathematical model and attempt to generate curve. This was first done by Michaelis and Menten for an equilibrium model. Better is the steady state model of Haldane and Briggs (more general), which we will derive.

For S ——›P assume

$$E + S \xrightleftharpoons[k_2]{k_1} [ES] \; (\xleftrightarrow{fast} EP) \xrightleftharpoons[k_4]{k_3} E + P$$

And for initial reaction conditions [P] = 0 & therefore k_4 = 0, so have

$$E + S \xrightleftharpoons[k_2]{k_1} [ES] \; (\xleftrightarrow{fast} EP) \xrightarrow{k_3} E + P$$

Now v_i = d[P]/dt = k_3[ES] (Note that k_{cat} is often used instead of k_3);

Assume steady state (steady state assumption: d[ES]/dt= 0):

d[ES]/dt= 0; Thus: 0 = d[ES]/dt= k_1[E][S] - k_2[ES] - k_3[ES].

Continuing we can now substitute for E (free enzyme), because hard to find, and gather constants:

$[E] = [E_t] - [ES]$; then

$d[ES]/dt = k_1([E_t][S] - [ES][S]) - k_2[ES] - k_3[ES]$,

gathering constants: $\frac{k_2 + k_3}{k_1} = \frac{[S]([E_t] - [ES])}{[ES]}$

Now define $\frac{k_2 + k_3}{k_1} = K_M$

Then $K_M = \frac{[S]([E_t] - [ES])}{[ES]}$, where K_M is the Michaelis-Menten constant.

{Note that if $k_2 >> k_3$ (that is the equil. of E+S with ES is rapid compared to breakdown of ES to P), then M-M const = 1/(affinity)= the dissociation constant, but only in these special conditions.}

Now a couple of tricks: Solve for [ES]: $[ES] = \frac{[E_t][S]}{K_M + [S]}$

and recall that $k_3[E_t] = V_{max}$ and therefore $v_i = k_3[ES]$, and dividing both sides by k_3, $v_i/k_3 = [ES]$

Substituting: $\frac{v_i}{k_3} = \frac{[E_t][S]}{K_M + [S]}$ and $v_i = k_3 \frac{[E_t][S]}{K_M + [S]}$,

But maximum possible velocity must = $k_3[E_t] = V_{max}$

So, $v_i = \frac{V_{max}[S]}{K_M + [S]} = \frac{V_{max}}{1 + K_M/[S]}$ Which is known as the ***Michaelis-Menten Equation.***

Hence, Michaelis - Menten constant, Km, is defined as the substrate concentration at half maximal velocity and is expressed as mole per litre (Fig. 6.2).

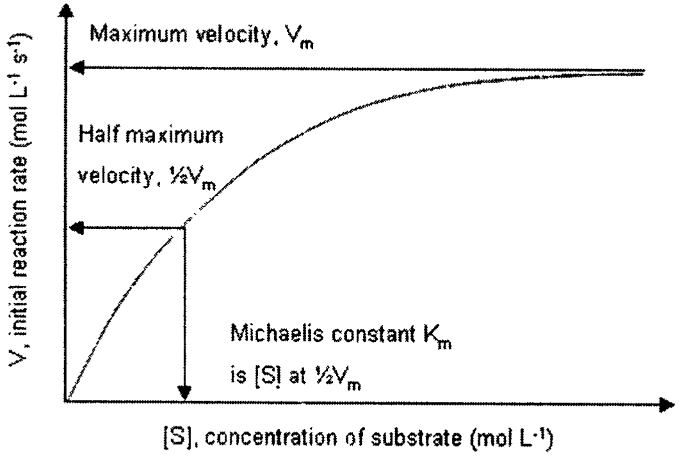


Fig. 6.2 : Graphical Presentation of Michaelis Menten Equation

For simple, one-substrate enzymes then, have *Michaelis-Menten Equation* as a model for enzyme activity.

$$v_i = \frac{V_{\max}[S]}{K_M + [S]} = \frac{V_{\max}}{1 + K_M/[S]}$$

Note predicted consequences of model:

- [S] >> K_M; then $v_i = V_{max}$ and get Zero order (r = k)
- [S] << K_M; then $v_i = \frac{V_{\max}}{K_M}[S]$, and get First order (r = k [S])
- [S] = K_M; then $v_i = V_{max}/2$ This is definition of K_M, the substrate concentration at half-saturation.
- Note consequences for a plot: start off with approximately linear slope with y = kx. Then at the limit of high concentrations have a horizontal line. This is exactly what we expect if we look at the general form of the equation: $y = \frac{ax}{x+b}$, the formula for a rectangular hyperbola (Fig. 6.3)

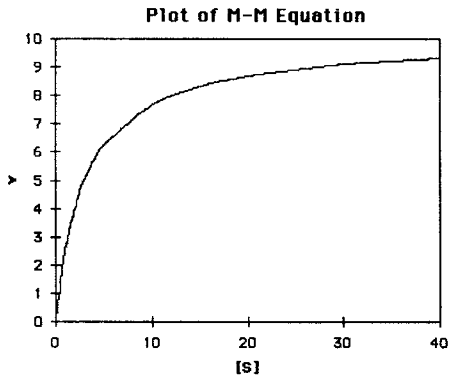


Fig. 6.3 : Hyperbolic curve

Turnover Number. The rate constant (First order) for the breakdown of the [ES] complex, k_{cat} (k_3), is also known as the *turnover number,* that is the maximum number of substrate molecules processed/active site (moles substrate/mole active site): $k_{cat} = V_{max} / [E]_{total}$. Note that this is best determined under saturating conditions. At very low concentrations of [S] can find the second-order rate constant for the conversion of $E + S \longrightarrow E + P$: $v_o = (k_{cat} / K_M)([E][S]$.

Linear Plots for Enzyme Kinetic Studies

The Michaelis-Menten equation can be algebraically transformed into more useful way to plot the experimental data. Lineweaver and Burk have taken the reciprocal of both [S] and v of the Michaelis-Menten equation to give

Double Reciprocal or Lineweaver-Burke Plot: Need in form: y = ax + b , so take reciprocals of both sides (Fig. 6.4) and have -

$$\frac{1}{v_i} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$$

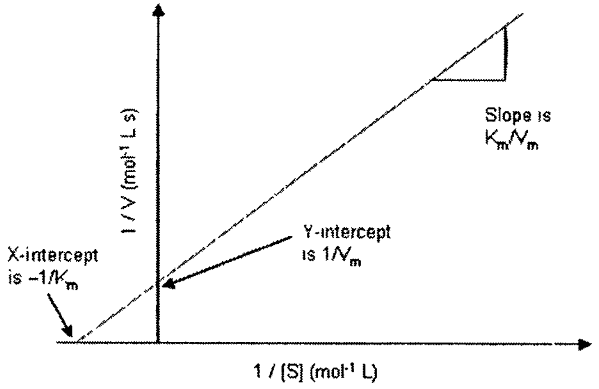


Fig. 6.4 : Double Reciprocal curve for Lineweaver-Burke Equation

A plot of 1/v versus 1/ [S] (the double reciprocal) yields a straight line. This line intercept X-axis at -1/Km and Y-axis at 1/Vmax. The slope of the line is Km/Vmax. The Lineweaver-Burk plot has the great advantage of allowing more accurate determination of Vmax and Km.

Significance of Km

i. Km value may vary with substrate.

ii. An enzyme whose Km is very low will have a high degree of affinity for its substrate (Table 6.2).

Table 6.2 : Km Values for some enzyme- substrate systems

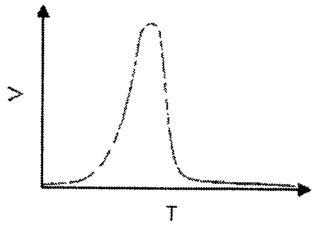
Enzyme	Substrate	Km(mM)
Catalase	H_2O_2	0.001
Hexokinase from brain	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO^{3-}	9
Chymotrypsin	N-Benzoyl tyrosinamide	2.5
	Glycyltyrosinylglycine	108
β-Galactosidase	Lactose	4.0
Pyruvate carboxylase	Pyruvate	1.0

Enzyme Concentration

When compared to substrate concentration, the concentration of enzyme is always very very low on molar basis. Hence, increasing the enzyme concentration will always increase the reaction rate.

Temperature

Over a limited range of temperature, the velocity of enzyme-catalysed reactions roughly doubles with a 10°C rise in temperature. Enzymes, being proteins, are denatured by heat and become inactive as the temperature increases beyond a certain point. Most of the enzymes are inactivated at temperatures above 60°C. The temperature at which the reaction rate is maximum is known as optimum temperature.



pH

Most enzymes have a characteristic pH at which their activity is maximum; (Fig. 6.5) above or below this pH, the activity declines. The pH affects the ionic state of the enzyme and frequently that of the substrate also. If a negatively charged enzyme (E-) reacts with a positively charged substrate (SH+), the ESH is formed. At low pH values, E- will be protonated and ESH is not formed. Similarly, at very high pH values SH+ will ionize and lose its positive charge.

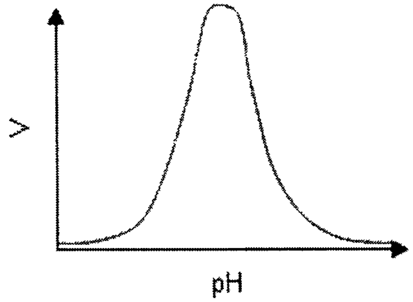


Fig. 6.5 : Relationship between pH and Velocity of Enzyme Reaction

$$E^- + SH^+ \dashrightarrow ESH$$

acidic pH

$E^- + SH^+ \dashrightarrow EH + SH^+ \dashrightarrow$ No ESH formation

alkaline pH

$SH^+ \dashrightarrow S + H^+ + E^- \dashrightarrow$ No ESH formation

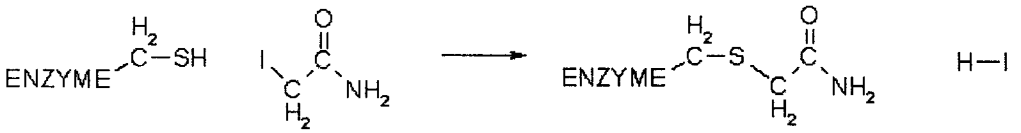
Another important factor is the change in conformation (denaturation) of enzyme at extreme pH values.

Inhibitors

Some compounds have the ability to combine with certain enzymes but do not serve as substrates and therefore block catalysis. These compounds are called inhibitors. The important type of inhibitors are described below:

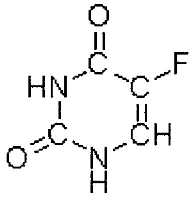
Irreversible Inhibitors

It binds to the enzyme and destroy the active site, or otherwise screw the protein. *Suicide inhibitors*, a special class of such inhibitors, are activated by the normal catalytic activity of the enzyme, but form an intermediate that binds to and destroys the active site. Irreversible inhibitors bind tightly (often covalently) to the enzyme and cannot be removed by dialysis. They include such things as nerve gases (Sarin, DIPF, Tabun) and insecticides (Malathion).



Iodoacetamide is the archetypal enzyme inhibitor: it indiscriminately binds to and deactivates cysteine residues.

Suicide inhibitors generally look like the substrate, but attack the enzyme when activated. 5-fluorouracil (which is converted in the body to 5F-dUMP) is a suicide inhibitor of thymidylate synthase, and prevents DNA synthesis in cancerous cells.



Because suicide inhibitors bind to the active site, the can be used to find what amino acids are present there. Malathion and other organophosphates are suicide inhibitors of insect AChE, so are widely used as insecticides.

Reversible Inhibitors

Two important subtypes - competitive and non-competitive inhibitors.

Competitive Inhibitor

In *competitive inhibition*, both inhibitor and substrate can bind to enzyme and form two independent complexes. Only ES degrades to products: EI is considered a 'dead-end'. Because the inhibitor binds, to the active site, the substrate cannot (and *vice versa*), so there cannot be an ternary ESI complex (Fig. 6.6).

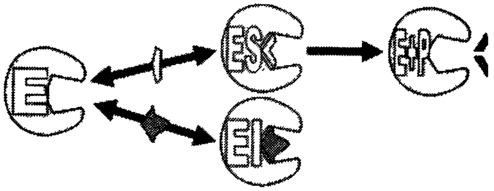


Fig. 6.6 : Models for Competitive Inhibition

Any compound which possessess a close structural resemblance to a particular substrate and which competes with that of substrate for the same active site on the enzyme is called as competitive inhibitor. The inhibitor is not acted upon by the enzyme and so remains bound to the enzyme preventing the substrate to bind. This is a reversible process. It depends upon the relative concentration of substrate and inhibitor. Competitive inhibition can be completely reversed by addition of large excess of substrate.

The enzyme, succinate dehydrogenase converts succinate to fumarate. For this reaction, malonic acid is a competitive inhibitor as it structurally resembles that of succinate.

Non-competitive Inhibitor

Non-competitive inhibitors reversibly to somewhere other than the active site; they change the protein conformation allosterically, and reduce the rate at which the enzyme turns over product. They have no effect on K_m as the active-site of uninhibited enzyme molecules will only encounter substrate, and no unproductive binding will occur. They *do* however reduce the apparent V_m; consequently the apparent V_m will be reduced, since the protein is no longer as enzymatically competent. Such inhibitors are generally not substrate analogues.

Because the inhibitor can bind independently of the substrate, an ESI complex can also form. Both ESI and EI are dead-ends (Fig. 6.7).

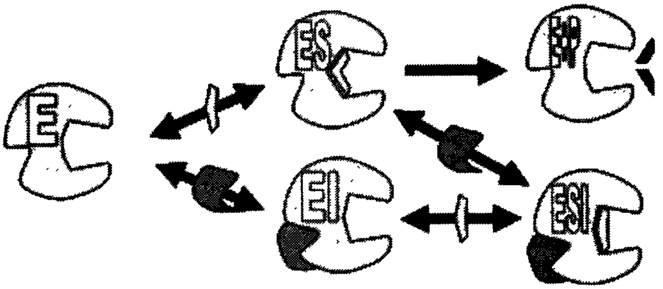


Fig. 6.7 : Model for Non-competitive Inhibitors

Non-competitive inhibitors bind to a site other than the active site on the enzyme often to deform the enzyme, so that, it does not form the ES complex at its normal rate. Once formed, the ES complex does not decompose at the normal rate to yield products. These effects are not reversed by increasing the substrate concentration.

Some enzymes possessing an essential -SH group are non-competitively inhibited by heavy metal ions (Hg2+, Pb2+). Some metalloenzymes are inhibited non competitively by metal chelating agents like ethylene diamine tetraacetic acid (EDTA).

Inhibitors are used as tools to probe the mechanism of enzyme - catalysed reactions and as therapeutic agents.

Isoenzymes

Enzymes which exist in multiple forms within a single species of organism or even in a single cell are called isoenzymes or isozymes. Such multiple forms can be detected and separated by gel electrophoresis of cell extracts. Since they are coded by different genes, they differ in amino acid composition and thus in their isoelectric pH values. Lactate dehydrogenase is an example for the isoenzymes which occur as five different forms in the tissues of the human and other vertebrates. All the five isozymes catalyze the same reaction.

$$\text{Lactate} + \text{NAD}^+ \longrightarrow \text{Pyruvate} + \text{NADH} + \text{H}^+$$

They have the molecular weight of about 134,000 and contain four polypeptides. The five isozymes consist of five different combinations of two different kinds of polypeptides M and H. Kinetic study of lactate dehydrogenase isozymes has revealed that although they catalyze the same reaction, they differ significantly in their Km values for their substrates as well as Vmax values. The two polypeptide chains in LDH are coded by two different genes. Skeletal muscle contains four identical M chains and designated as M4; whereas heart muscle contains four identical H chains and designated as H4. LDH of other tissues are a mixture of the five possible forms H4, H3M, H2M2, HM3 and M4. A determination of the relative amounts of the five LDH isozymes and the total concentration of LDH in a serum sample can provide valuable diagnostic information about which tissues have been damaged and the extent of the damage.

Enzyme Regulation

Metabolism involves the tight integration of many complex pathways. The regulation of these pathways is mostly achieved by the regulation of enzymes. This is particularly important in major metabolic pathways (glycolysis, Krebs, urea cycle, gluconeogenesis, *etc.*), which impact important (and energetically expensive) pathways. Enzymes are frequently regulated, in contrast to inorganic catalysts, which generally cannot be regulated. Enzymes that are regulated usually stand at crossroads of metabolic pathways, and have a small k_{cat} (they are slow), so they rarely run quickly enough to catalyse their reactions to equilibrium: Q (mass action ratio, [products] = [reactants]) = K_{eq}. They often work in pairs, catalysing slightly different reactions in the forward and backwards directions (Fig. 6.8).

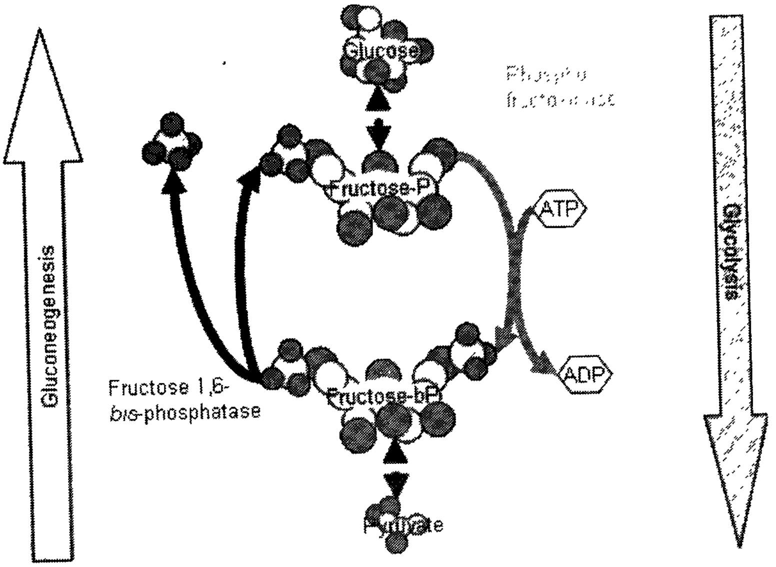


Fig. 6.8 : Model for Enzyme Regulation

Phosphofructokinase is often claimed to be the gatekeeper to glycolysis: it is the step that (more-or-less) irrevocably commits fructose-6-phosphate to conversion to pyruvate (and hence to Krebs). Although this may be over-egging the pudding, it is certainly highly regulated and requires ATP. Fructose-1,6-*bis*-phosphatase appears to catalyse the backwards reaction, but this is not quite correct: it converts fructose-1,6-*bis*-phosphate to fructose-6-phosphate, but by dephosphorylation: it does *not* regenerate ATP. This two-enzyme system allows glycolysis and gluconeogenesis to be tightly regulated.

The rate at which regulation can be effectively achieved varies greatly, from hours to microseconds. In approximate order of speed, enzymes may be regulated by:

1. Regulation by genetic expression of enzyme (slowest). There is always competition in a cell between the processes of protein synthesis and protein destruction. By altering these rates, one can alter the whole cell catalytic rate. However, it is rather slow, although proteins with a high turnover rate will respond more quickly.
2. Compartmentation of substrate and enzyme. Enzymes can also be compartmentalised, like the hydrolytic enzymes found in the lysosome, but the release of these suicide enzymes during apoptosis is rather more of an on/off switch than a true regulation.

3. Activation of a zymogen. Some enzymes are secreted as inactive precursors, called *zymogens.* Trypsin and pepsin are two such examples: a portion of the zymogen must be cleaved off to form the active enzyme. Again, an on/off switch more than a tight, variable regulation.
4. Reversible phosphorylation or adenylation. Enzymes can be phosphorylated on their tyrosine, threonine or serine residues. This is a very common regulatory strategy, and a common end-product of a signal cascade.
5. Competitive product inhibition and allosteric regulation (fastest). Many enzymes are inhibited by either their products, or by other chemicals, often those from further down a metabolic pathway. Such enzymes may be 'gatekeepers' to a specific branch of metabolism, and they usually catalyse a true equilibrium reaction, *i.e.,* one that doesn't go to completion (note this is not exactly the same reaction in the forward and backwards directions, so we are not defying the law that states enzymes do not alter the equilibrium point).

There will be looking principally at the last three ways.

In negative feedback, the later or final products of a metabolic sequence feed-back negatively on early steps, *e.g.,* in Krebs cycle, the final product of a metabolic sequence feeds-back negatively on early steps (Fig 6.9).

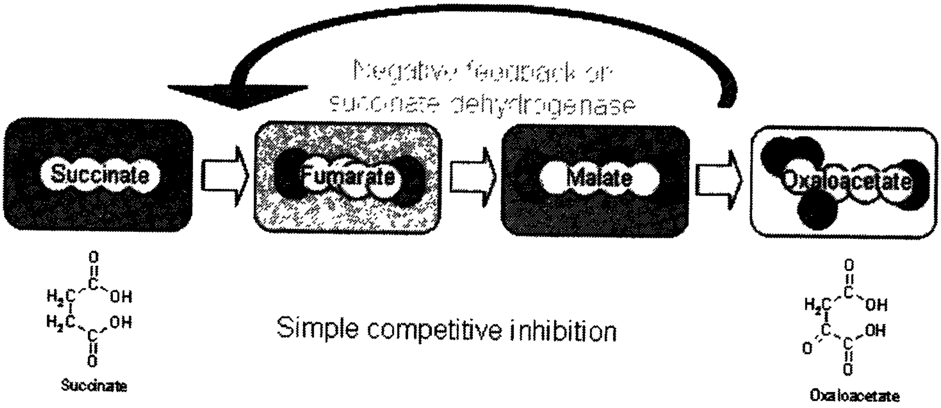


Fig. 6.9 : Negative Feed back Inhibition

In positive feedforward, earlier reactants in a metabolic sequence feed-forward positively on later steps. If A is accumulating, it makes sense to speed up downstream reactions to use it up, *e.g.,* fructose-1,6-*bis*phosphate activates pyruvate kinase in glycolysis. A combination of feedback and feedforward is used to regulate enzyme activity (Fig. 6.10).

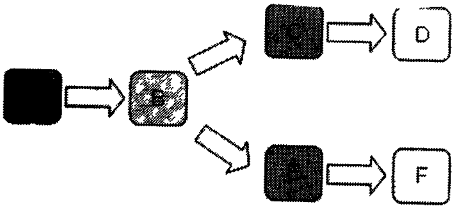


Fig. 6.10 : Positive Feed forward Inhibition.

Metabolism involves the complex integration of many feedback and feedforward loops.

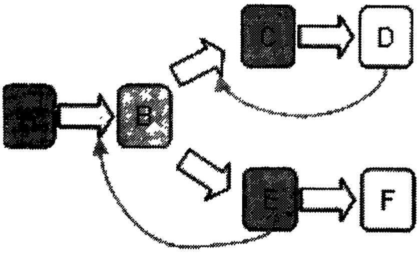


Fig. 6.11 : Integration of many Feedback and Feef forward Inhibition

However, there is a problem here: it is unlikely that D, A and F are similar to B, so they cannot inhibit by simple competition, and how on earth can an inhibitor positively regulate a reaction anyway? So what happens instead?

The regulation of enzymes by metabolites leads to the concept of *allosteric regulation*. Allosteric means 'other structure'. Allosteric modulators can bind at a site other than the active site in question and cause activation *or* inhibition. These modulators can include the substrate itself, which binds at *another* active site in a multi-subunit enzyme. In fact, allosterically modulated enzymes almost always have a complex quaternary structure (multiple subunits) and exhibit non-Michaelis-Menten kinetics.

The enzyme phosphofructokinase (PFK) is regulated by:

- High concentrations of ATP, which inhibit PFK
- High concentrations of ADP, which stimulate PFK
- High concentrations of AMP, which stimulate PFK
- High concentrations of citrate, which inhibit PFK

Therefore, ATP and citrate are allosteric inhibitors; and ADP and AMP are allosteric activators (Fig. 6.12).

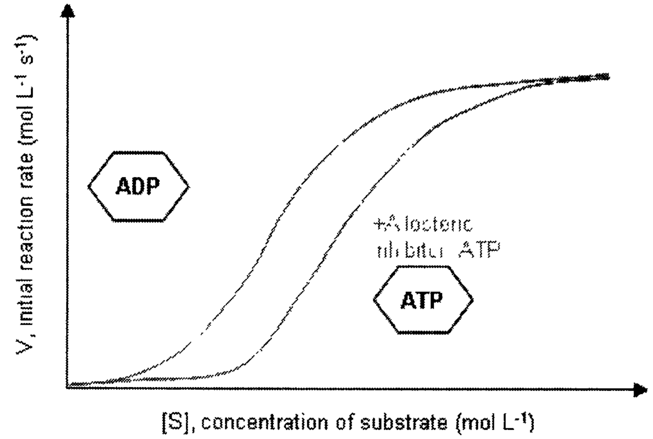


Fig. 6.12 : Sigmoidal Curves for Allosteriz Enzyme Regulation

Why the sigmoid shape? Allosteric enzymes are multi-subunit enzymes, each with an active site. They show a cooperative response to substrates as well as to modulators, and their affinity for substrate increases with increasing substrate concentration. This means that V increases rapidly over a small range of [S] values, then plateaus off rapidly.

Haemoglobin is a four subunit protein (although it is not an enzyme) that binds oxygen, and is often used as a model for allosteric regulation because it is a good model for cooperation in the binding of multiple ligands. Myoglobin is a closely related monomeric protein. The % saturation with oxygen is equivalent to the saturation of the active site of an enzyme: myoglobin shows normal saturation kinetics; but haemoglobin shows sigmoid kinetics (Fig 6.13).

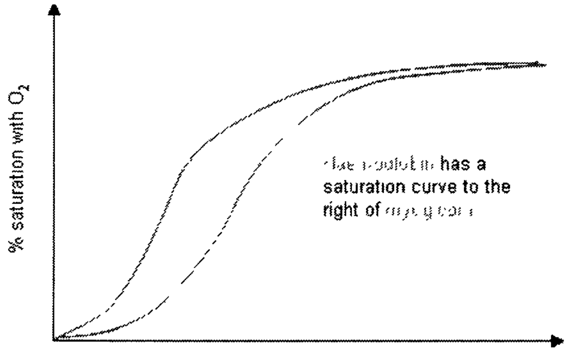


Fig. 6.13 : Comparison of Allosteric Regulation for Haemoglobin and Myoglobin

Binding constant, $K_b = [Hb(O_2)_{n+1}] / [Hb(O_2)_n]\ [O_2]$.

Table 6.3 : Equilibrium constant values for Haemoglobin (Hb).

Equilibrium constant	Equation	Value
K_{b1}	$[Hb(O_2)] \longrightarrow [Hb][O_2]$	0.024
K_{b2}	$[Hb(O_2)_2] \longrightarrow [Hb(O_2)][O_2]$	0.074
K_{b3}	$[Hb(O_2)_3] \longrightarrow [Hb(O_2)_2][O_2]$	0.083
K_{b4}	$[Hb(O_2)_4] \longrightarrow [Hb(O_2)_3][O_2]$	7.4

O_2 molecules bind sequentially to Hb with binding constants K_{b1} to K_{b4}, and you can see that each O_2 binds *more* tightly than the last. The differences in K_b cannot be explained on the basis that each subunit has a different binding constant: if this was the case the subunit with the highest K_b would bind first, in practice it binds last (Fig. 6.14).

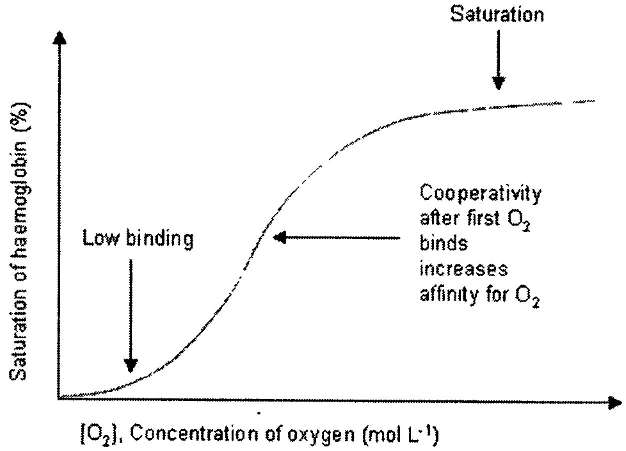


Fig. 6.14 : O2 Affinity Curves for Haemoglobin (Hb)

Oxygen binds to the most *difficult* site first. This alters the conformation of the protein so the next subunit binds oxygen more easily. This process is then repeated for the other two subunits. This change in conformation caused by the progressive binding of a ligand is known as cooperativity and leads to sigmoid kinetics. The binding of one ligand (substrate or modulator) changes the conformation of the protein. This can increase *or* decrease the affinity for further ligands. There are two models for this cooperativity:

- Concerted model (Wyman, Monod & Changeux).
- Sequential model (Koshland).

Both models recognise that subunits change their conformation and alter the binding constant (K_b) for ligands. The low affinity form is known as tense (T).

The high affinity form is relaxed (R), and these exist in equilibrium with each other.

In the sequential model, each binding of substrate increases the affinity of the other active sites (Fig 6.15).

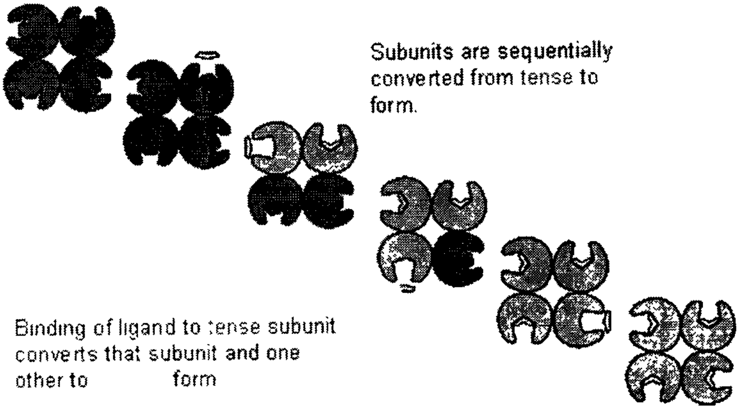

Subunits are sequentially converted from tense to form.
Binding of ligand to tense subunit converts that subunit and one other to form

Fig. 6.15 : Sequential Model for Enzyme substrate Affinity

In the concerted model, the first binding of substrate increases the affinity of the other active sites, but further bindings have no effect. This doesn't contradict the haemoglobin/oxygen binding constants presented earlier, as the increased affinities at ever increasing oxygen concentrations could be due to the increased proportion of relaxed form, rather than to individual increases in each protein molecule (Fig. 6.16).

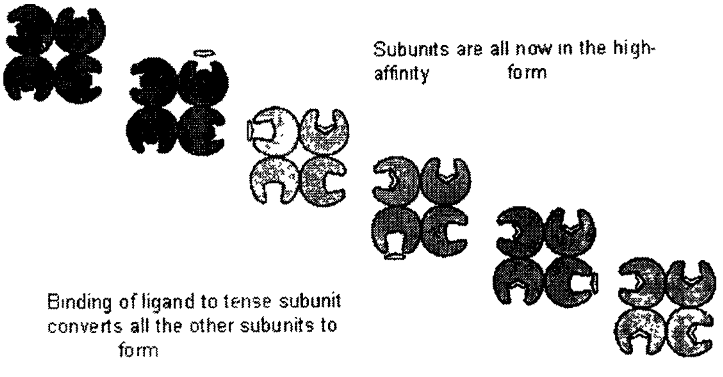

Subunits are all now in the high-affinity form
Binding of ligand to tense subunit converts all the other subunits to form

Fig. 6.16 : Concerted Model for Enzymes-substrate Affinity

The currently accepted model for allosteric inhibitors and activators is based on the concerted model. Inhibitors lock all subunits in the tense form, whereas activators locks all subunits in the relaxed form.

In allosteric inhibition, the inhibitor locks the enzyme in the tense conformation. Binding of substrate (when sufficiently little inhibitor is present) locks the enzyme in the relaxed form (Fig. 6.17).

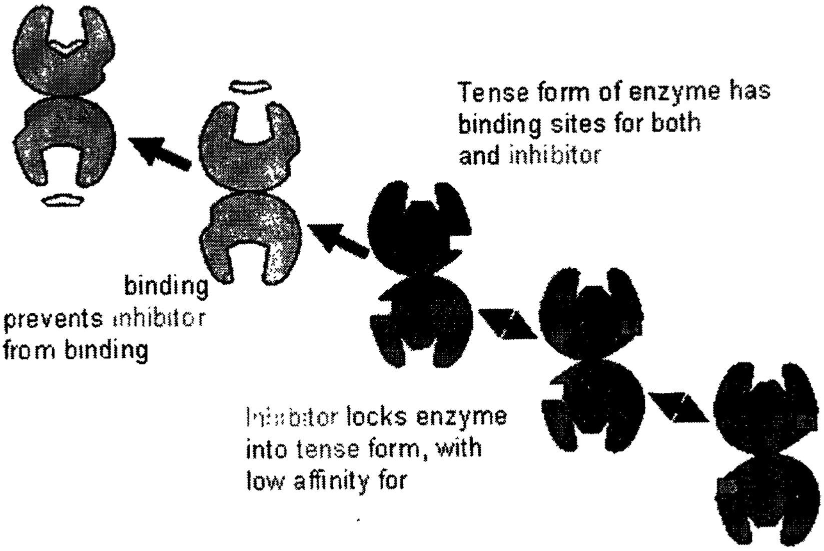

Tense form of enzyme has
binding sites for both
and inhibitor
binding
prevents inhibitor
from binding
Inhibitor locks enzyme
into tense form, with
low affinity for

Fig. 6.17 : Allosteric Inhibition

In allosteric activation, the activator locks the enzyme in the relaxed conformation (Fig. 6.18).

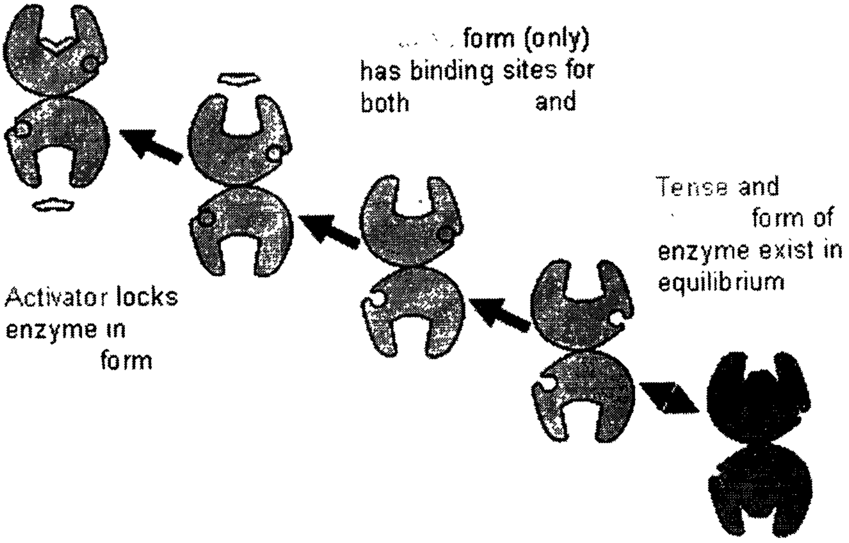

form (only)
has binding sites for
both and
Tense and
form of
enzyme exist in
equilibrium
Activator locks
enzyme in
form

Fig. 6.18 : Allosteric Activation

There is some vocabulary you should know for cooperativity:

- Positive: one molecule facilitates the binding of another.
- Negative: one molecule makes the binding of another more difficult.
- Homotropic: one molecule influences the binding of a second similar molecule.
- Heterotropic: one molecule influences the binding of a different molecule.

A typical allosteric inhibitor therefore cooperates in a heterotropic, negative fashion.

Phosphorylation is another very common way of regulating enzymes, especially in signalling cascades. It requires ATP. A frequently quoted example is glycogen phosphorylase, an enzyme that phosphorylates glycogen, and is itself most active when phosphorylated. Phosphorylation of glycogen phosphorylase is reversible and controlled by the phosphorylase kinase and a phosphatase. (kinases add phosphate groups to proteins, phosphatases remove them). The phosphorylase kinase is itself regulated by phosphorylation (Fig. 6.19).

The regulation is rather complex:

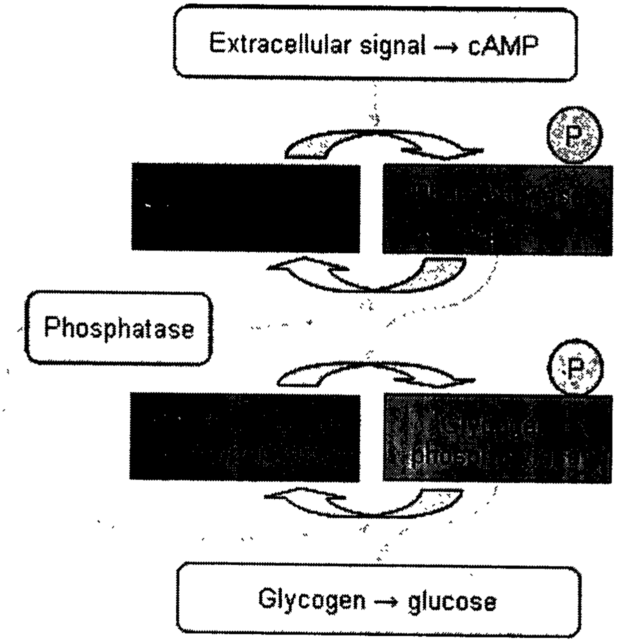


Fig. 6.19 : Enzyme Regulation through Phosphorylation

Protein kinases like glycogen-phosphorylase kinase act on specific amino-acid sequences, called consensus sequences. Protein-kinase-a acts on the serine/threonine residue in the sequence X-arg-arg/lys-X-ser/thr-asx. Cyclin-dependent-kinase-2 acts on the same residues in the sequence X-ser/thr-pro-X-lys/arg. Only the alcoholic amino acids serine, threonine and tyrosine can be phosphorylated.

Zymogens are the final way of regulating enzymes. Zymogens are inactive enzyme precursors. They are activated by external means, and undergo cleavage to produce an active enzyme. Digestive enzymes are mostly regulated in this way, because we don't want them to digest the cell that synthesises them! *e.g.*, Pepsin is activated by H^+ in the stomach. Trypsin and chymotrypsin follow a more complex scheme (Fig. 6.20).

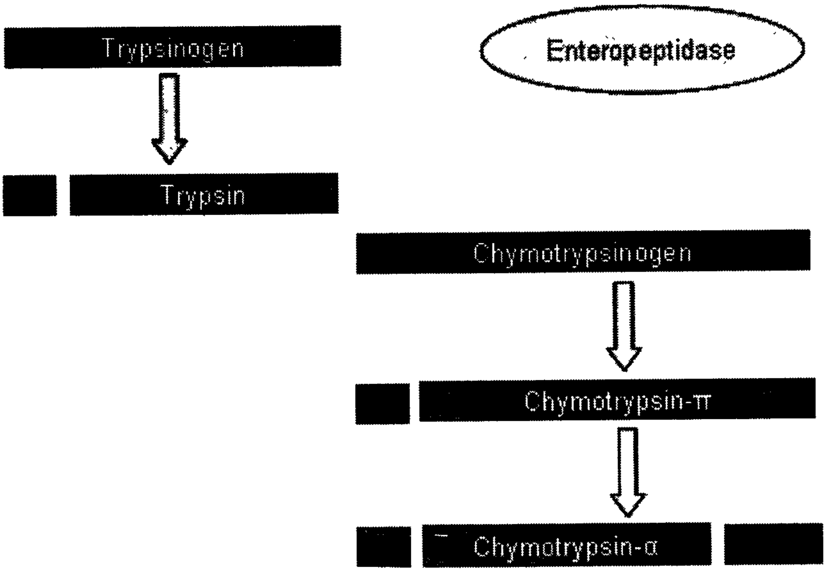


Fig. 6.20 : Trypsin and Chymotrypsin Regulation through Zymogens

QUIZ

Give Answer in brief

Q-1 What is the importance of enzymes in living systems?

A-1 Enzymes are the reaction catalysts of the biological systems. They have extraordinary catalytic power, often greater than that of synthetic catalysts. They have high degree of substrate specificity & accelerate specific chemical reactions. They function in an aqueous medium under very mild conditions of temperature& pH. Very few non-biological catalysts show all these properties.

Q-2 If enzymes are not present in body what will happen?

A-2 The biochemical reactions taking place in living cells at body temperature at sufficiently rapid in a regular order. Such reactions would have been extremely slow, had they not been catalyzed by enzymes, which are present in every living cell and can also act independently of the cell. (e.g. hydrolysis of starch by salivary amylase).

Q-3 Were enzyme actions were known in earlier time?

A-3 Yes, Biological catalysis was first recognized & described in the early 1800 in studies of digestion of meat by secretions of stomach & conversion of starch into sugar by saliva & various plant extracts. In 1850 Louise Pasteur concluded that fermentation of sugar into alcohol by yeast is catalyzed by" FERMENTS "& later named than enzymes, are inseparable from living yeast cells, a view that prevailed for many years.

Q-4 What was Buchner's important discovery regarding enzyme? (Or fermentation?)

A-4 In 1897 Buchner showed yeast extract (cell free) could ferment sugar to alcohol. This encouraged biochemists to attempt the isolation of many different enzymes & to examine their catalytic properties.

Q-5 What was the important discovery made by James Sumner?

A-5 In 1926 James Sumner crystallized UREASE and found it is as a protein and postulated that all enzymes are proteins. This idea remained controversial for some time.

Q-6 What was contribution of J. B. S. HALDANE regarding enzyme?

A-6 During the period 1930, J. B. S. HALDANE wrote a treatise entitled enzymes, even though molecular nature of enzymes was not fully yet appreciated. It contained remarkable suggestions of weak bonding interactions and substrate catalyzed reaction.

Q-7 What is an enzyme?

A-7 Enzymes are the reaction catalysts of the biological systems. They are protein in nature (With exception of small group of catalytic RNA molecules). Molecular weight ranges from 12000 to over million. They are specific in action e.g. Urease. Their catalytic activity depends upon the integrity of their native protein conformation. If enzyme is denatured or dissociated into subunits catalytically activity is usually lost. The enzymes carry out transformation of molecules and also mediate transformation of energy e.g. PHOTOSYNTHESIS.

Q-8 What are simple and complex enzymes?

A-8 Some enzymes require no chemical groups other than amino acids for activity, Enzymes composed of only protein are known as simple enzymes.

Complex enzyme composed of protein plus a relatively small organic molecule, which is required for enzyme activity.

Q-9 What is apo-enzyme?

A-9 Apo-enzyme is the protein part of an enzyme without any cofactors or prosthetic group that may be required for the enzyme to be functional. The apo-enzyme is catalytically inactive.

Q-10 What is coenzyme or prosthetic group?

A-10 A non-protein component of an enzyme, which is required for catalytic activity, is known as co-enzyme or prosthetic group.

Q-11 What is co-factor?

A-11 Is small organic or inorganic molecules that an apo-enzyme requires for its activity. e.g. in lysine oxidase, copper is loosely bound which act as co-factor.

Q-12 What is the difference between prosthetic group and co-enzyme?

A-12 A coenzyme or metal ion that is covalently bound to the enzyme protein is called prosthetic group. For e.g. in the cytochromes, the haeme prosthetic group is very tightly bound and requires strong acids to dissociate from its apo enzyme.

Q-13 Do some Enzymes contain vitamin derivatives?

A-12 Yes, Many prosthetic groups and coenzymes are water-soluble derivatives of vitamins. It should be noted that the main clinical symptoms of dietary vitamin insufficiency generally arise from the malfunction of enzymes, which lack sufficient cofactors derived from vitamins to maintain homeostasis.

Q-14 What is a holo enzyme?

A-13 A complete catalytically active enzyme together with its coenzyme and/or metal ions called a holoenzyme. Apo-enzyme + Co enzyme == Holoenzyme.

Q-14 What is metalloenzyme and metal activated enzymes?

A-14 Enzymes require a metal in their composition (such as Fe^{+2},$Mg^{2+\cdot}$ Mn^{2+},Zn^{2+}) are known as metalloenzymes. If they bind and retain their metal atom(s) under all conditions, that is with very high affinity. Those, which have a lower affinity for metal ion, but still require the metal ion for activity, are known as metal-activated enzymes.

Q-15 How enzymes are named?

A-15 Many enzymes have been named by adding the suffix "-ase" to the name of their substrate or to a word or phrase describing their activity. e.g. UREASE catalyze hydrolysis of urea, MALTASE act on maltose, and DNA polymerase catalyze the synthesis of DNA. Other enzymes such as PEPSIN and TRYPSIN have names they do not denote their substrates.

Q-16 What is IUB system?

A-16 Sometimes the enzyme may have two or more names, or two different enzymes have the same name. Because of such ambiguities, and ever increasing number of newly discovered enzymes a system for naming and classifying enzymes has been adopted by inter national agreement, International Union of Biochemistry (IUB-system)

Q-17 How enzymes are classified according to IUB system?

A-17 According to IUB system enzymes are grouped in six major classes. Each with sub classes based on the type of reaction catalyzed. Systemic classification of enzymes based on numbering system is used. Each enzyme is assigned a code number or EC (enzyme commission number) four-digit classification number and a systematic name, which identifies the reaction catalyzed.

Q-18 According to IUB system which enzymes are included in group 1?

A-18 OXIDO-REDUCTASE (EC-1)

Enzymes of this group add or remove hydrogen atoms during the catalysis. They include dehyrogenases & oxidases, and are mostly concerned with biological oxidation.

Dehydrogenases removes H+ from substrate in the presence of H+ acceptor group. e.g. Lactate Dehydrogenase. Enzymatic action can be represented by:

$AH_2 + B$ ------ à $A + BH_2$

OXIDASES – transfer two electrons from the donor to oxygen resulting usually in hydrogen peroxide formation (H_2O_2) e.g. Glucose oxidase. CYTOCHROME oxidase produces H_2O rather than H_2O_2. Oxygenases catalyze the incorporation of oxygen into a substrate.

Q-19 According to IUB system which enzymes are included in group 2?

A-19 TRANSFERASES (EC-2)

These enzymes transfer functional groups between donors and acceptors. The AMINO, ACYL, PHOSPHATE, ONE CARBON and GLYCOSYL are the major groups that are transferred. e. g. A— X+ B ------- à A+ B—X

A. METHYL group---àe.g. Transmethylase
B. ALDEHYDE or KETONIC group e.g. Transaldolase or transketolase.
C. ACYL GROUP e.g.Aceyltransferase
D. SUGAR GROUP e.g. Glucosyltransferase
E. AMINO-KETO GROUP- Aminotransferase or transaminases
F. KINASES are specialized trnsferases that regulate metabolism by transferring phosphate from ATP to other moleculs e.g. Hexokinase: ATP + Glucose ----- G-6-P+ ADP

Q-20 According to IUB system which enzymes are included in group 3?

A-20 HYDROLASES (EC-3)

A special class of transferases in which the donor group is transferred to water. The generalized reaction involves the hydrolytic cleavage of C-O, C-N, O-P and C-S bonds. In other words enzymes, which add, water to the substrate and hydrolyze or decompose it to give products. A – B + H_2O ------ AH+ BOH

1. LIPASES------- e.g. Glycerol ester hydrolase
2. PHOSPHATASES------e.g.Glucose-6-Phosphatase
3. CHOLINE ESTERASE hydrolysases acetylcholine
4. PEPTIDASES-----hydrolyses peptides
5. NUCLEASES e.g.nucleotidase, nucleosidase
6. CARBOHYDRASES e.g. Amylase act on amylose Lactase, Maltase
7. Enzymes acting on C – N linkage - Urease converts urea into ammonia, Asparginase, Glutaminase, Arginase

Q-21 According to IUB system which enzymes are included in group 4?

A-21 LYASES (EC4)

Lyases add or remove water, ammonia, or carbon dioxide from the substrates.

A-B + X-Y ------- A-X + B-Y

1. DECARBOXYLASE removes CO2 from a orb keto acids or aminoacids.
2. Carbonic anhydrase
3. Cysteine desulfurase

Q-22 According to IUB system which enzymes are included in group5?

A-22 ISOMERASES (EC5)

A hetrogeneous group of enzymes catalyze transfer of groups within molecules to yield isomeric forms e.g. isomerazition of! (1) Optical isomers, (2) geometrical isomers.

A----→ A′

1. Epimerases or Racemases catalyze inversion at asymmetric carbon atoms
2. MUTASES involve intramolecular transfer of a group such as a phosporyl
3. CIS-TRANS ISOMERASE e.g. all trans retinene isomerase

Q-23 According to IUB system which enzymes are included in group 6?

A-23 LIGASES (EC6)

To liagate means to bind, Formation of C-C, C-S, C-O and C-N bonds by condensation reactions. These enzymes carry out synthetic reactions where two molecules joined at the utilization of a "high energy phosphate bond of ATP. "

REACTION : A+B --- → A-B

1. Pyruvate carboxylase, Pyruvate + CO_2 + ATP----- → Oxaloacetate + ADP + Pi
2. GLUTAMINE SYNTHETASE

3. Acetyl Co A CARBOXYLASE

Q-24 The following enzyme is classified as EC -2.7.1.1., What does this mean?

A-24 The name of the enzyme catalyzing following reaction is ATP:glucose phospo transferase. Its enzyme classification number (E.C.number) is 2.7.1.1

ATP + D-Glucose -------- → D-Glucose-6-phosphate+ ADP

The first digit (2) denotes the class number (transferase)

The second digit (7) denotes sub-class - phospo transferase;

The third digit (1)-Hydroxyl group as an acceptor;

The fourth digit (1) D-glucose as the phosphate group acceptor.

When the systematic name of an enzyme is long or cumbersome, a *trivial* name may be used e. g. Hexokinase.

Identify the letter of the choice that best completes the statement or Answers the question.

1. Enzymes are catalysts that:
   A. accelerate the rates of biological reactions.
   B. inhibit the formation of unwanted metabolites.
   C. change the equilibrium constants of reactions.
   D. are generally non-specific in reactions they catalyze.

Answer: A

2. The turnover number of an enzyme is the number of:
   A. enzyme-substrate complexes formed per unit time.
   B. substrate molecules converted to product before the enzyme is saturated.
   C. substrate molecules converted into product per enzyme molecule per unit time, when the enzyme is saturated with substrate.
   D. product molecules formed from an enzyme-substrate complex per unit time.

Answer: C

3. In the presence of a pure non-competitive inhibitor:
   A. The Km is increased. B. The Km is decreased.
   C. The Vmax is increased. D. The Vmax is decreased.

Answer: D

4. If a plot of the reciprocal of the velocity of an enzyme catalyzed reaction versus the reciprocal of the substrate concentration yields a straight line, the intercept on the 1/V axis is:
   A. V/Km B. -1/Km
   C. Km/Vmax D. 1/Vmax

Answer: B

5. For an enzyme reaction obeying Michaelis-Menten kinetics the horizontal part of a v versus [S] plot is indicative of:
   A. a first-order reaction with respect to substrate
   B. a second-order reaction with respect to substrate
   C. a mixed-order reaction with respect to substrate
   D. a zero-order reaction with respect to substrate

Answer: D

6. One of the basic assumptions of the concerted model of the allosteric behavior of proteins is that in the absence of ligand:
   A. The two states (R and T) of the allosteric protein are in equilibrium.
   B. There are equal concentrations of the R and T states of the allosteric protein.
   C. The R state of the allosteric protein is always present in greater concentrations than the T state.
   D. The T state of the allosteric protein is always present in greater concentrations than the R state.

Answer: A

7. For an enzyme that obeys simple Michaelis-Menten kinetics, if a plot of the reciprocal of the substrate concentration against the reciprocal of the initial velocity yields a straight line, then the intercept on the 1/v axis is:
   A. v/Km B. -1/Km
   C. Km/Vmax D. 1/Vmax

Answer: C

8. A non-competitive inhibitor of an enzyme catalyzed reaction causes:
   A. an increase in both the apparent Km and Vmax
   B. a decrease in the apparent Km and Vmax
   C. no change in the apparent Km and a decrease in apparent Vmax
   D. an increase in the apparent Km and no change in apparent Vmax

Answer: C

9. The enzyme glycoprotein peptidase is irreversibly inhibited by penicillin, which forms a covalent adduct with a serine residue on the enzyme. From this observation which statement logically follows?
   A. Serine must be protonated for this enzyme to be active.
   B. All enzymatic reactions require an intact functional serine residue.
   C. Serine may be involved in binding of the substrate.
   D. The catalytic mechanism of this enzyme involves the transient formation of a penicillin-enzyme adduct.

Answer: C

10. In the presence of a competitive inhibitor the initial rate of an enzyme catalyzed reaction depends upon:
    A. the substrate concentration

B. the inhibitor concentration
C. the relative affinities of inhibitor and substrate
D. all of the above factors

Answer: D

11. Enzymes accelerate thermodynamically possible reactions by:
A. altering the equilibrium constant
B. lowering the activation energy of the reaction
C. lowering the overall free energy change for the reaction
D. reducing the temperature coefficient

Answer: B

12. Heterotropic effectors of allosteric proteins:
A. always influence the protein to form more of the R state
B. always influence the protein to form more of the T state
C. always cause increased binding of the substrate to an enzyme
D. influence the binding of something other than themselves to the allosteric protein

Answer: D

13. Diisopropylfluorophosphate (DIFP) irreversibly inactivates proteases such as trypsin and chymotrypsin by reacting with:
A. the carboxyl side chain of a glutamic acid residue
B. the coenzyme
C. the N-terminal amino acid
D. the hydroxyl group of a serine residue

Answer: D

14. A number of the digestive enzymes are synthesized in an inactive form and are only activated in the duodenum. These inactive precursor forms are termed:

A. zymogens	B. substrates
C. coenzymes	D. trypsins

Answer: A

15. In the serine proteases, the formation of an acyl-enzyme intermediate is indicative of:
A. the susceptibility of such enzymes to inhibition by diisopropylfluorophosphate
B. the participation of an aspartic acid residue at the active site
C. the involvement of a covalent attachment to the enzyme as part of the catalytic process
D. an active-site tryptophan residue e. a competitive inhibitor

Answer: C

16. The compounds diisopropylfluorophosphate (DIFP) is a potent inhibitor of the proteolytic enzyme chymotrypsin. It permanently inactivates the

enzyme by forming a covalent complex with a _____ residue at the active site

A. serine B. proline
C. histidine D. lysine

Answer: A

17. The inhibition of chymotrypsin by diisopropylfluorophosphate (DIFP) involves:
    A. the transient electrostatic interaction of the inhibitor with positively charged residues at the active site
    B. reversible competitive inhibition of substrate binding
    C. irreversible inactivation by the formation of a covalent complex with an active-site histidine
    D. the formation of a covalent enzyme-inhibitor complex at the active-site serine

Answer: D

18. One of the reasons that enzymes are such efficient catalysts is that:
    A. The energy level of the enzyme-transition state complex is much higher than for an uncatalyzed reaction.
    B. Enzymes can lower the activation energy for the reaction.
    C. The translational entropy of the substrate is greatly increased upon binding to the enzyme.
    D. The enzyme typically binds the substrate much more strongly than the transition state.

Answer: B

19. Which of the statements regarding enzymes is false?
    A. Enzymes are proteins that function as catalysts.
    B. Enzymes are specific.
    C. Enzymes provide activation energy for reactions.
    D. Enzyme activity can be regulated.

Answer: C

20. The relationship between an enzyme and a reactant molecule can best be described as:
    A. a temporary association.
    B. an association stabilized by a covalent bond.
    C. one in which the enzyme is changed permanently.
    D. a permanent mutual alteration of structure.

Answer: A

21. When [S] = KM, the velocity of an enzyme catalyzed reaction is about:
    A. 0.1*Vmax.
    B. 0.2*Vmax.

C. 0.3*Vmax.
D. 0.5*Vmax.

Answer: D

22. The active site of an enzyme
A. remains rigid and does not change shape.
B. is found at the center of globular enzymes.
C. is complementary to the rest of the molecule.
D. None

Answer: D

23. The active site of an enzyme differs from an antibody-antigen binding site in that the enzyme active site
A. contains modified amino acids.
B. catalyzes a chemical reaction.
C. is complementary to a specific ligand.
D. contains amino acids without sidechains.

Answer: B

24. The transition state of a catalyzed reaction (EX‡) is
A. a highly-populated intermediate on the reaction pathway.
B. higher in energy than that of an uncatalyzed reaction.
C. lower in energy than that of an uncatalyzed reaction.
D. lower in energy than the reaction substrate.

Answer: C

25. The substrate KM in an enzyme-catalyzed reaction
A. is usually less than Kd, the dissociation constant.
B. is never less than KD.
C. cannot be equal to KD.
D. is estimated from the Y-intercept of a Lineweaver-Burk plot.

Answer: B

26. The initial velocity, vo, of an enzyme catalyzed reaction reaches Vmax
A. at [S] = KM.
B. at [S] = 10*KM.
C. at 1/[S] = 1/KM.
D. only as 1/[S] --> 0.

Answer: D

27. When [S] = 0.1*KM, the velocity of an enzyme catalyzed reaction is about:
A. 0.1*Vmax.
B. 0.3*Vmax.
C. 0.5*Vmax.
D. 0.7*Vmax.

Answer: A

28. When [S] = 10*KM, the velocity of an enzyme catalyzed reaction is about:

A. 0.9*Vmax.
B. 0.3*Vmax.
C. 0.5*Vmax.
D. 0.7*Vmax.

Answer: A

29. A competitive inhibitor of an enzyme is usually:
A. a highly reactive compounD.
B. a metal ion such as Hg2+ or Pb2+.
C. structurally similar to the substrate.
D. water insoluble.

Answer: C

30. An uncompetitive inhibitor of an enzyme catalyzed reaction
A. binds to the Michaelis complex (ES).
B. decreases Vmax.
C. is without effect at saturating substrate concentration.
D. The first and second choices are both correct.

Answer: D

31. Which of the following common drugs is not a specific enzyme inhibitor?
A. methotrexate.
B. penicillin.
C. sulfonilamide.
D. iodine.

Answer: D

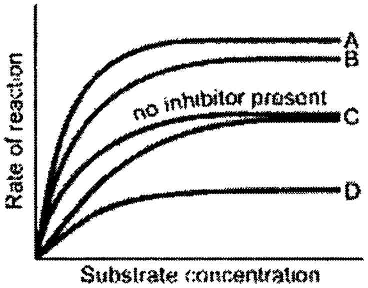
32. A molecule of acetylcholinesterase normally hydrolyzes about 1,000 molecules of acetylcholine each seconD. After reacting with a nerve gas such as sarin, the hydrolysis rate of this enzyme would be about
A. 1,000/sec.
B. 100/sec.
C. 10/sec.
D. 0/sec.

Answer: D

33. An allosteric inhibitor of an enzyme usually
A. binds to the active site.
B. participates in feedback regulation.
C. denatures the enzyme.
D. causes the enzyme to work faster.

Answer: B

34.

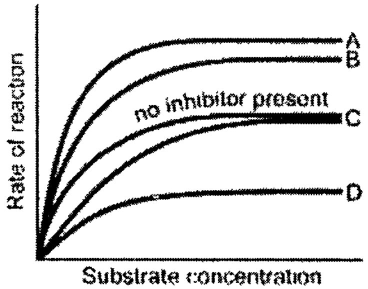


In the diagram, which letter represents the 'non-active site-directed inhibitor present' curve:

A. A B. B C. C D. D

Answer: D

35.



In the diagram, which letter represents the 'active site-directed inhibitor present' curve:

A D B. C C. B D. A

Answer: B

36. Which of the following is not a commercial advantage of enzyme immobilization:
A. the reaction can be carried out at higher temperatures
B. the initial cost of the enzyme is reduced
C. the thermal stability of the enzyme is increased
D. enzyme easily recovered from reaction mixture

Answer: B

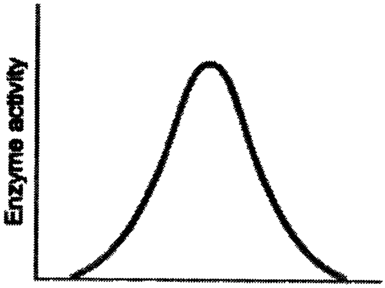
37. Enzymes used to break down proteins in biological washing powders belong to the group:
A. lactases B. proteases
C. hydrolases D. lipases

Answer: B

38. Chemicals (other than the substrate) that affect enzyme activity are called:
A. inhibitors
B. exhibitors

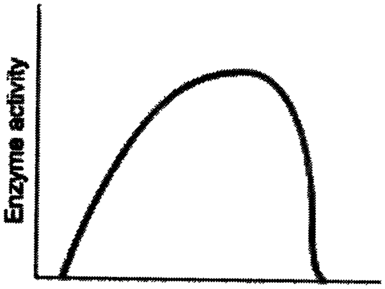
C. mobilizers
D. immobilizers

Answer: A



39. The diagram shows a typical relationship between enzyme activity and:
A. temperature
B. substrate concentration
C. enzyme concentration
D. pH

Answer: D



40. The diagram shows a typical relationship between enzyme activity and:
A. temperature
B. substrate concentration
C. enzyme concentration
D. pH

Answer: A

41. Enzymes speed up biochemical reactions by:
A. increasing the activation energy of the reaction
B. lowering the activation energy of the reaction
C. lowering the temperature of the reaction
D. increasing the temperature of the reaction

Answer: B

42. Enzymes belong to which group of chemicals:
A. polysaccharides
B. lipids

C. saccharides
D. proteins

Answer: D

43. Which of the following enzymes would digest a fat?
A. sucrase B. fatase C. protease D. lipase

Answer: D

44. At high temperatures, the rate of enzyme action decreases because the increased heat
A. changes the pH of the system
B. alters the active site of the enzyme
C. neutralizes the acids and bases in the system
D. increases the concentration of the enzyme

Answer: B

45. Enzymes influence chemical reactions in living systems by
A. providing the substrate required for the reaction to occur
B. affecting the rate at which reactions occur
C. absorbing water released when polymers are formed
D. combining with excess hydrogen to form gaseous wastes

Answer: B

46. Which group of organic compounds includes the enzymes?
A. proteins B. starches C. carbohydrates D. lipids

Answer: A

47. The "lock and key hypothesis" attempts to explain the mechanism of
A. vacuole formation
B. pinocytosis
C. sharing of electrons
D. enzyme specificity

Answer: D

48. Any substance that is acted upon by an enzyme is called a(n)
A. coenzyme B. substrate C. vitamin D. polypeptide

Answer: B

49. An enzyme that hydrolyzes protein will not act upon starch. This fact is an indication that enzymes are
A. hydrolytic B. specific C. catalytic D. synthetic

Answer: B

50. At 25 C. the optimum reaction rate of a certain enzyme occurs at a pH of 7. A greater reaction rate could probably be attained by
A. increasing the temperature to 35 C and keeping the pH at 7
B. increasing both the temperature and the pH
C. decreasing the pH and increasing the temperature
D. increasing the pH and keeping the temperature at 25 C.

nswer: A

51. At about 0 C., most enzymes are

A. inactive B. active C. destroyed D. replicated

Answer: A

52. Vitamins are essential to the survival of organisms because vitamins usually function as

A. substrates B. nucleic acidsC. coenzymes D. nucleotides

Answer: C

53. Which element is present in maltase, but not in maltose?

A. carbon B. hydrogen C. oxygen D. nitrogen

Answer: D

54. A certain enzyme will hydrolyze egg white but not starch. Which statement best explains this observation?

A. Starch molecules are too large to be hydrolyzed.
B. Enzyme molecules are specific in their actions.
C. Egg white acts as a coenzyme for hydrolysis.
D. Starch is composed of amino acids.

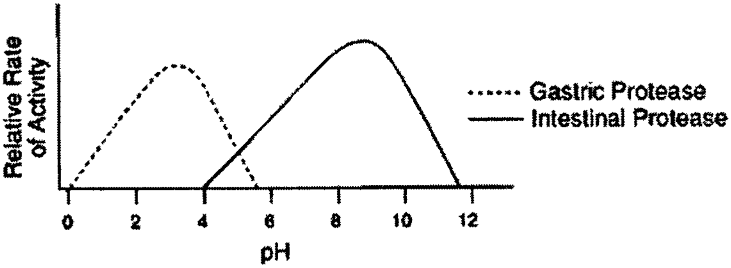
Answer: B

55. Which environmental condition would most likely have the LEAST effect on the rate of enzyme controlled hydrolytic reactions in humans?

A. the pH of the solution
B. the temperature of the solution
C. the amount of enzyme present
D. the amount of light present

Answer: D

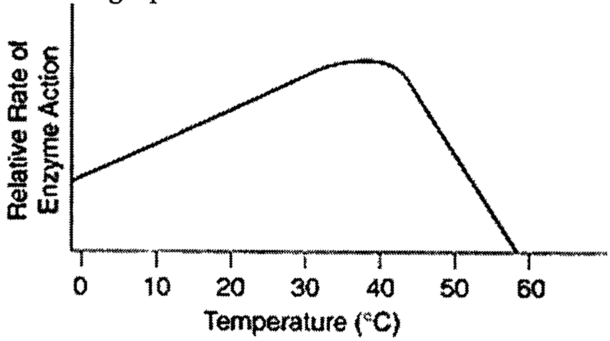
56. Which statement best expresses the information represented in the graph shown?



A. The action of enzymes varies with pH.
B. A pH of 7 provides the optimum environment for digestive enzymes
C. Gastric juice is active at a pH extending from 0 to 12.
D. Acids have a pH greater than 7.

Answer: A

57. The effect of temperature on the relative rate of action of an enzyme is represented in the graph below.



The optimum temperature for the action of this enzyme is approximately
A. 15 C B. 22 C C. 37 C D. 50 C

Answer: C

Complete each sentence or statement.

1. A/An ________ is a noncovalently bound small organic molecule that is required for the catalytic activity of an enzyme.

Answer: coenzyme

2. A/An ________ is a compound containing an electron-rich group.

Answer: nucleophile

3. The bond that is cleaved during a hydrolytic reaction is the ________ bond.

Answer: scissile

4. An RNA molecule that has catalytic activity is a/an ________.

Answer: ribozyme

5. ________ is the reverse of condensation.

Answer: Hydrolysis

6. A compound that bears a negative charge on a carbon atom is a/an ________.

Answer:carbanion

7. Covalent catalysis is a catalytic mechanism in which the transient formation of a covalent bond between the catalyst and a reactant lowers the free energy of a reaction's ________.

Answer: transition state

8. ________ is a technique for identifying functional groups in a macromolecule by treating the molecule with a reagent that reacts with those groups.

Answer: Chemical labeling

9. ________ is the process of forming a blood clot.

Answer: Coagulation

10. Different proteins that catalyze the same reaction are called ________.

Answer: isozymes

11. During the process of ________, it appears that the compound catalyzes its own activation because the product of the activation reaction also acts as a catalyst for the same reaction.

Answer: autoactivation

12. A Schiff base is an ________ that forms between an amine and an aldehyde or ketone.

Answer: imine

13. The hydrogen bonded Ser, His, and Asp residues that participate in catalysis in serine proteases make up the ________.

Answer: catalytic triad

14. ________ is the ability of an enzyme to discriminate between possible substrates and to catalyze a single type of chemical reaction.

Answer: Reaction specificity or Substrate specificity

15. The region of an enzyme in which catalysis takes place is called the ________.

Answer: active site

16. ________ is a chronic disease characterized by difficulty breathing due to alveolar degeneration and loss of lung elasticity.

Answer: Emphysema

17. A/An ________ reacts readily with a nucleophile.

Answer: electrophile

18. The independent development of similar characteristics in unrelated species is called ________.

Answer: convergent evolution

19. ________ is a peptide-hydrolyzing enzyme that has a reactive Ser residue in its active site.

Answer: Serine protease

20. A substance that promotes a chemical reaction without undergoing permanent change is a/an ________.

Answer: catalyst

21. The phenomenon in which an enzyme's conformation changes upon binding with a substrate is called ________.

Answer: induced fit

22. A/An ________ is a short, strong hydrogen bond in which the proton is shared equally by the donor and acceptor atoms.

Answer: low-barrier hydrogen bond

23. An affinity label is a labeled compound that resembles an enzyme's __________ but reacts irreversibly with and thereby labels a group in the enzyme's active site.

Answer: substrate

24. ________ is a catalytic mechanism in which sequestering the reacting groups away from the aqueous solvent lowers the free energy of a reaction's transition state.

Answer: Electrostatic catalysis

25. Tautomers are isomers that differ only in the position of a/an ________ atoms.

Answer: hydrogen

26. The rate of a/an ________ reaction is proportional to the square of the concentration of one reactant or to the product of the concentrations of two reactants.

Answer: second-order

27. The noncovalent complex that forms between an enzyme and a reversible inhibitor is a/an ________.

Answer: EI complex or enzyme-inhibitor complex

28. The use of information about an enzyme's structure, mechanism, and inhibitors to design even more effective inhibitors is called ________.

Answer :rational drug design

29. A/An ________ is a polypeptide that undergoes proteolysis after its synthesis to yield several separate protein molecules.

Answer: polyprotein

30. A molecule that chemically inactivates an enzyme only after undergoing part of the normal catalytic reaction is a/an ________.

Answer: suicide substrate

31. Michaelis-Menten equation is a mathematical expression that describes the activity of an enzyme in terms of the ________, the enzyme's maximal velocity, and its Michaelis constant.

Answer: substrate concentration

32. The catalytic constant (kcat) is also known as the ________.

Answer: turnover number

33. The ________ is a mathematical expression for the time-dependent progress of a reaction as a function of reactant concentration.

Answer: rate equation

34. The upper limit for the rate of a second-order reaction in solution is the ________.

Answer: diffusion-controlled limit

35. A/An ________ is a substance that inhibits the activity of an enzyme that catalyzes an early step of the substance's synthesis.

Answer: feedback inhibitor

36. In ________, binding of an activator to one subunit of a multisubunit enzyme increases the catalytic activity of all the subunits.

Answer: allosteric activation

37. ________ is a state achieved by an enzyme that operates at the diffusion-controlled limit.

Answer: Catalytic perfection

38. A/An ________ reaction involves two molecules.

Answer: bimolecular

39. A negative effector is a substance that diminishes an enzyme's activity through ________.

Answer: allosteric inhibition

40. A/An ________ is a molecule that binds to and permanently inactivates an enzyme.

Answer: irreversible inhibitor or inactivator

41. A/An ________ is the noncovalent complex that forms between an enzyme and its substrate in the first step of an enzyme-catalyzed reaction.

Answer: ES complex or enzyme-substrate complex

42. A stable substance that geometrically and electronically resembles the transition state of a reaction and that therefore may inhibit an enzyme that catalyzes the reaction is a/an ________.

Answer: transition state analog

43. Product inhibition is a form of enzyme inhibition in which the reaction product acts as a/an ________.

Answer: competitive inhibitor

44. Under ________ conditions, the formation and degradation of individual components are balanced such that the system does not change over time.

Answer: steady state

45. ________ is a form of enzyme inhibition in which an inhibitor binds to the enzyme such that it affects both Michaelis constant and maximal velocity.

Answer: Mixed inhibition

“This Page is Intentionally Left Blank”

Chapter 7

Vitamins and Coenzymes

Vitamins are low molecular weight organic compounds required in small amounts in the diet. Most of the vitamins are not synthesized in the human body but are synthesized by the plants. Hence these essential nutrients are mainly obtained through the food. Though most of them are present in the diet as such, some are present as precursors known as provitamins.

Vitamins are divided into two major categories. They are fat-soluble (A, D, E and K) and water-soluble vitamins (B-complex and vitamin C). B complex vitamins include thiamine (B_1), riboflavin (B_2), pantothenic acid (B_3), niacin (B_5), pyridoxine (B_6), biotin (B_7), folic acid (B_9), and cobalamin (B_{12}). Inositol, cholic and para-aminobenzoic acid are vitamin-like substances sometimes classified as part of the B complex, but no convincing evidence has been shown so far to be included as vitamins. All the fat-soluble vitamins and some B vitamins exist in multiple forms. The active forms of vitamin A are retinol, retinal and retinoic acid and vitamin D is available as ergocalciferol (D_2) and cholecalciferol (D_3). The vitamin E family includes four tocopherols and four tocotrienols but α-tocopherol being the most abundant and active form. The multiple forms of vitamins are interconvertible and some are interchangeable.

Fat-Soluble Vitamins

The fat-soluble vitamins are soluble in fat and other nonpolar solvents. All are synthesized fully or partly from isoprene units and excess quantities are stored in fat containing cells. The fat-soluble vitamins appear not to function as components of coenzymes but to serve other important roles. The important dietary sources, functions and deficient diseases associated with fat- soluble vitamins are given in Table 7.1.

Table 7.1 : Sources, Functions and Deficiency symptoms of Fat Soluble Vitamins

Vitamin	Functions	Some common dietary sources	Deficiency symptoms
Vitamin A	Visual cycle and maintaining epithelial cells	Fruits, vegetables, fish-liver oils	Night blindness and eventually total blindness, anorexia (appetite loss), dermatitis, recurrent infections; in children, cessation of skeletal growth and lesions in the central nervous system.

Vitamin D	Calcium metabolism	Fish-liver oil	Bone pain and skeletal. deformities such as bowlegs. (Rickets) and knock-knee in children. Osteomalacia in adults.
Vitamin E	Antioxidant	Plant oils, green leafy vegetables, milk, eggs, meat	Symptoms in humans, if any, are controversial; possibly anaemia.
Vitamin K	Blood clotting	Leafy vegetables, soybeans, vegetable oils	Impaired blood clotting.

Water-Soluble Vitamins

The water-soluble vitamins include B-complex group and vitamin C. The important dietary sources and deficient symptoms associated with them are given in Table 7.2.

Table 7.2 : Sources of Water soluble vitamins and their Deficiency symptoms

Vitamin	Some common dietary sources	Deficiency symptoms in humans
Thiamine (Vitamin B_1)	Liver, meat, milk, vegetables, whole grains, nuts	Dry and wet beri-beri. Weight loss-, muscle wasting, sensory changes, mental confusion, enlargement of heart, constipation.
Riboflavin (Vitamin B_2)	Liver, wheat germ, eggs, milk, green leafy vegetables, meat	Magenta-coloured tongue, fissuring at the corners of mouth and lips, dermatitis.
Pantothenic acid (Vitamin B_3)	Eggs, peanuts, liver, meat, milk, cereals, vegetables	Vomiting, abdominal distress, cramps, fatigue, insomnia.
Niacin or nicotinic acid (Vitamin B_5)	Meat, liver, cereals, legumes	Pellagra. Dermatitis when exposed to sunlight, weakness, insomnia, impaired digestion, diarrhea, dementia, irritability, memory loss, headaches.
Pyridoxine or pyridoxol (Vitamin B_6)	Egg yolk, fish, meat, lentils, nuts, fruits, vegetables	Convulsions, dermatitis, weight loss, irritability, weakness in infants.
Biotin (Vitamin B_7)	Liver, yeast, meat, peanuts, eggs, chocolate, dairy products, grains fruits, vegetables	Dermatitis, skin dryness, depression, muscle pain, nausea, anorexia (appetite loss).

Folic acid (Vitamin B_9)	Yeast, liver, green vegetables, some fruits	Anaemia leading to weakness, tiredness, sore tongue, diarrhea, irritability, headache, heart palpitations.
Cobalamin (Vitamin B_{12})	Meat, shellfish, fish, milk, eggs	Neurological disorders, anemia leading to tiredness, sore tongue, constipation, headache, heart palpitations.
Ascorbic acid (Vitamin C)	Vegetables and citrus fruits	Sore gums, loose teeth, joint pain, edema, anaemia, fatigue, depression, impaired iron absorption, impaired wound healing.

Coenzymes

Mechanism of Coenzyme Action

Coenzyme accelerates the enzymatic reaction by helping the formation of the product (s) by acting as acceptor for one of the products.

The substrate combines with the apoenzyme to form activated complex. But this combination takes place in the presence of coenzyme. The bond in the substrate is strained and ruptured when one of the cleavage products is directly transferred to the coenzyme, which has suitable receptor site in its structure. The other cleavage product now dissociates from the apoenzyme liberating the enzyme protein for fresh reaction. The cleavage product attached to the coenzyme is next released from the surface of the coenzyme after the completion of enzyme action. Now both apoenzyme and coenzyme are regenerated to their original form and are ready for fresh reaction. A prosthetic group also acts in a similar fashion with the difference that the prosthetic group is firmly attached to the surface of the apoenzyme.

The structure and coenzyme functions of B-complex group vitamins are described below.

Thiamine

The two important reactions in which TPP functions as coenzyme are :

- Oxidative decarboxylation of α-keto acids such as pyruvate and α -ketoglutarate.
- Transketolase reaction.

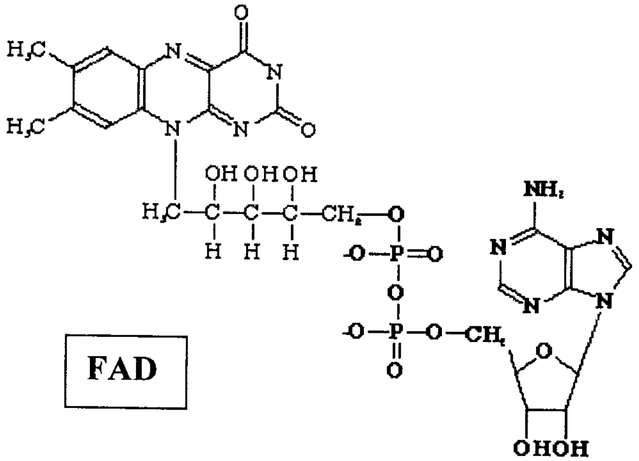
TPP provides a reactive carbon on the thiazole ring and forms a carbanion stabilized by positively charged ring nitrogen. The carbanion is then free to add the carbonyl carbon of pyruvate (or α-ketoglutarate). The addition compound is then decarboxylated eliminating CO2 and generating hydroxyethyl-TPP. This reaction occurs in a multienzyme complex known as pyruvate dehydrogenase complex (or α-ketoglutarate dehydrogenase complex). The acetaldehyde (decarboxylated product) moiety is then transferred to the lipoamide in the complex.

The role of TPP as a coenzyme in the transketolase reaction is very similar to that of oxidative decarboxylation. The carbanion of TPP combines with the carbonyl carbon of xylulose 5P. Carbon 1 and 2 of xylulose 5P are retained to form hydroxyethyl derivative of TPP. Then it is transferred to the carbonyl carbon of ribose 5P to form sedoheptulose 7P.

Riboflavin

The flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) are the two-coenzyme forms of riboflavin.

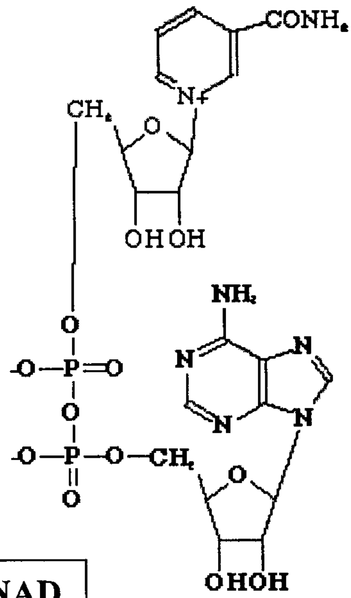
FMN and FAD serve as prosthetic groups of oxidation-reduction enzymes known as flavoenzymes or flavoproteins. They are usually tightly, but not covalently, bound to the protein. Many flavoproteins contain one or more metals as additional cofactors and are known as the metalloflavoproteins. In the catalytic cycle of flavoproteins the flavin moiety of the flavin nucleotides undergoes reversible reduction of the isoalloxazine ring to yield the reduced nucleotides FMNH2 and FADH2. L-amino acid oxidase contains tightly bound FMN as the prosthetic group. Succinate dehydrogenase and D-amino acid oxidase contain FAD as prosthetic group.



FAD

Niacin

The coenzyme forms of niacin are NAD+ and NADP+ which function as the coenzymes of a large number of oxidoreductases collectively called as pyridine-linked dehydrogenases. These coenzymes are bound to the dehydrogenase protein relatively loosely during the catalytic cycle and therefore serve as substrate than as prosthetic group. They function as electron acceptors during the enzymatic removal of hydrogen atoms from specific substrate molecules. One hydrogen atom from the substrate is transferred as a hydride ion to the nicotinamide portion of the oxidised forms of these coenzymes. The other hydrogen atom from the substrate becomes a hydrogen ion (Figure 8.5). Pyridine linked dehydrogenases are specific for either NAD+ or NADP+, but a few will function with both. Isocitrate dehydrogenase and lactate dehydrogenase are NAD-specific. Glucose 6-P dehydrogenase is NADP-specific. Glutamate dehydrogenase functions with NAD+ or NADP+.



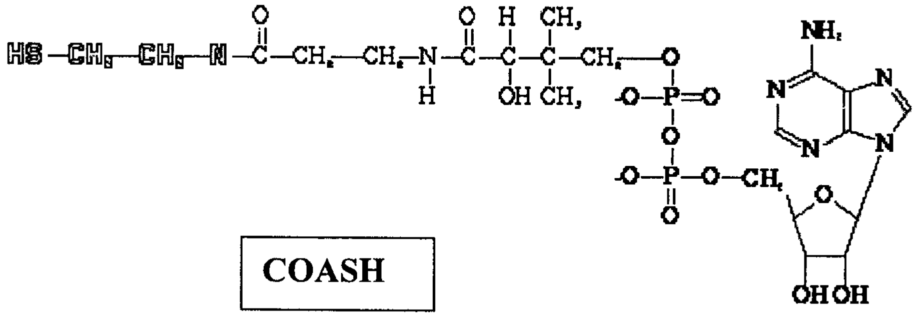
NAD

Pyridoxine

The coenzyme form of pyridoxine is known as pyridoxal phosphate (PP) The most common type of reaction requiring PP as a coenzyme is transamination. Enzymes catalysing such reactions are known as transaminases or aminotransferases. The coenzyme binds to its apoenzyme via Schiff's base between its aldehyde group and the epsilon amino group of a lysine in the enzyme. Additional ionic bond is also formed between its phosphate and the enzyme. During reaction, α-amino group of amino acid displaces the epsilon amino group forming a new Schiff's base. By a series of electron shifts and rearrangements, the pyridoxal phosphate becomes pyridoxamine phosphate. The amino acid is oxidatively deaminated to form the corresponding α-keto acid. The α-amino group is then transferred to a new α-keto acid to change it to an amino acid. PP also acts as coenzyme in the decarboxylation, desulphuration, transulphuration reactions associated with amino acid metabolism.

Pantothenic Acid

The coenzyme form of pantothenic acid is coenzyme A and is represented as CoASH. The thiol group (-SH) acts as a carrier of acyl group. The acyl-sulphur bond formed between coenzyme A and the acyl moiety is a high-energy bond, equivalent to the high-energy bond of ATP. The function of coenzyme A is to serve as a carrier of acyl group in reactions associated with fatty acid oxidation, fatty acid synthesis, pyruvate oxidation and biological acetylations. It is also involved in many biosynthetic processes such as synthesis of cholesterol, terpenes and steroids.



Biotin

The important function of biotin is its role as coenzyme for carboxylase, which catalyses carbon dioxide fixation or carboxylation reaction. The epsilon amino group of lysine in carboxylase enzymes combines with the carboxyl group of biotin to form covalently linked biotinyl carboxyl carrier protein (BCCP or biocytin) (Figure 6.8). This serves as an intermediate carrier of carbon dioxide. The carboxylation of acetyl CoA to malonyl CoA in presence of acetyl CoA carboxylase requires biotin as coenzyme. Propionyl carboxylase and pyruvate carboxylase are also associated with biotin.

Folic Acid

The coenzyme form of folic acid is tetra hydro folic acid (Figure 6.9). Tetrahydro folic acid is associated with one carbon metabolism. The tetrahydro folic acid serves as a carrier of single carbon moieties such as formyl, methenyl, methylene, formyl or methyl group. (Figure 6.10). It is involved in the biosynthesis of purines, pyrimidines, serine, methionine and glycine.

Lipoic Acid

The oxidised and reduced forms of lipoic acid are given in figure 6.11. Lipoic acid functions as a coenzyme in pyruvate and α-ketoglutarate dehydrogenase multienzyme complexes.

Cobalamin

The 5-deoxyadenosyl cobalamin and methyl cobalamin function as coenzyme forms and are required for the action of several enzymes. Methyl malonyl CoA mutase uses 5-deoxyadenosyl cobalamin as coenzyme. Methyl cobalamin functions as a carrier of methyl group to homocysteine and convert it to methionine

The functions of important coenzymes and their precursors are given in Table. 7.3

Table 7.3 : Functions of important coenzymes and their Precursors

Coenzyme	Short Form(s)	Chemical Groups Transferred	Vitamin Precursor
Thiamine pyro-phosphate	TPP	Two carbon aldehydes	Thiamine
Flavin adenine dinucleotide	FAD	Electrons	Riboflavin
Flavin mono nucleotide	FMN		
Nicotinamide adenine dinucleotide	NAD	Hydride ion	Nicotinic acid
Nicotinamide adenine dinucleotide Phosphate	NADP		
Coenzyme A	CoASH	Acyl group	Pantothenic acid
Pyridoxal phosphate	PP	Amino group	Pyridoxal (Pyridoxamine)
Coenzyme B12	Cobamide	Hydrogen atoms and alkyl groups	Vitamin B12
Biocytin	BCCP	Carbon dioxide	Biotin
Tetrahydrofolate	THF (FH4)	One-carbon groups	Folate
Lipoamide	-	Electrons and acyl groups	Lipoate

Vitamins are classified as fat-soluble and water-soluble. Fat-soluble vitamins include vitamins A, D, E and K. B-complex group and vitamin C belong to water-soluble vitamins. B-complex group of vitamins function as coenzymes and play a key role in biochemical reactions. The coenzyme form .of thiamine is thiamine pyrophosphate (TPP). It is associated with oxidative decarboxylation of α-keto acids such as pyruvate and α-ketoglutarate as well as transketolase enzyme. The carbanion formed in the thiazole ring of TPP condenses with the carbonyl carbon - pyruvate and then decarboxylation occurs. The flavin mononucleotide (FMN) and flavin adnine dinucleotide (FAD) are the two coenzymes forms of riboflavin. FMV and FAD serve as coenzymes for contain and oxidation reaction and under reversible reduction of the isoalloxazine ring to yield FMNH2 and FADH2. The coenzyme forms of niacin are NAD+ and NADP+, which function as the coenzymes for a large number of oxidoreductases. They function as electron acceptors. One hydrogen atom from the substrate is transferred as a hydride ion to the nicotinamide portion of the oxidized forms of these coenzymes. The coenzyme form of pyridoxine is pyridoxal phosphate. It function as coenzyme in transamination reaction as well as decarboxylation, desulphuration reaction in amino acid metabolism.

The coenzyme form of pantothenic acid is coenzyme A and is represented as CoASH. The thiol group acts as a carrier of acyl group. It is an important coenzyme involved in fatty acid oxidation, pyruvate oxidation and is also biosynthesis of terpenes. The epsilon amino group of lysine in carboxylase enzymes combines with the carboxyl carrier protein (BCCP or biocytin) and serve as an intermediate carrier of CO2. Acetyl CoA pyruvate and propionyl carboxylayse require the participation of BCCP. The coenzyme form of folic acid is tetrahydro folic acid. It is associated with one carbon metabolism. The oxidised and reduced forms of lipoic acid function as coenzyme in pyruvate and α-ketoglutarate dehydrogenase complexes. The 5-deoxy adenosyl and methyl cobalamins function as coenzyme forms of vitamin B12. Methyl cobalamin is involved in the conversion of homocysteine to methionine.

QUIZ

Give Answer in brief

Q-1 What are vitamins ?

A-1

- Vitamins are organic compounds found in foods and are essential in small quantities for the normal health and growth of the body.
- The distinguishing feature of the vitamins is that they generally cannot be synthesized by mammalian cells and, therefore, must be supplied in the diet.

- The most prominent function is as cofactors for enzymatic reactions.
- There are thirteen organic compounds known to function as vitamins in humans.
- Vitamins are classified into two categories: Fat-soluble Vitamins (A, D, E, K) and Water-soluble Vitamins (All B, Biotin, Folic acid and Ascorbic acid).
- There is minimal tissue storage of water soluble vitamins in the body, therefore, water soluble vitamins are less likely to accumulate to toxic levels than are fat-soluble vitamins. In the case of an overdose of vitamins, fat-soluble vitamins are potentially more toxic than water-soluble vitamins.
- The absence of one or more vitamins from the diet or poor absorption of vitamins can cause vitamin deficiency diseases.

Fat soluble vitamins

Q-2 Describe pro and active forms of Vitamin A.

A-2 Vitamin A consists of three biologically active molecules, retinol, retinal (retinaldehyde) and retinoic acid. Each of these compounds are derived from a group of molecules know as carotenoids also referred to as the provitamin A. Beta-carotene, which consists of two molecules of retinal linked at their aldehyde ends.

Q-3 Describe in brief, physico-chemical properties of Vitamin A.

A-3 It is a yellow color oil, insoluble in water but soluble in alcohol, chloroform etc. It consists of beta ionone ring with side chain of long chain unsaturated alcohol. It is unstable in the presence of heat, light, acid and oxidation while stable in alkaline media and also upon reduction.

Q-4 Describe absorption, storage and transport of Vitamin A.

A-4

- Ingested beta-carotene is cleaved in the lumen of the intestine by *beta-carotene dioxygenase* to yield retinal.
- Retinal is reduced to retinol by *retinaldehyde reductase,* an NADPH requiring enzyme within the intestines.
- Retinol esters present in the diet are hydrolyzed in the intestinal mucosa, releasing retinal and free fatty acids.
- Retinol is esterified to palmitic acid in the intestinal mucosa and secreted as components of chylomicrons into the lymphatic system and through blood stored in liver.
- The uptake of chylomicron remnants by the liver results in delivery of retinol to this organ for storage as a lipid ester within lipocytes.
- Transport of retinol from the liver to extra-hepatic tissues occurs by binding of hydrolyzed retinol to retinol binding protein (RBP).

- The retinol-RBP complex is then transported to the cell surface within the Golgi and secreted.
- Within extra hepatic tissues retinol is bound to cellular retinol binding protein (CRBP).
- Plasma transport of retinoic acid is accomplished by binding to albumin. Zinc is required for mobilization of vit. A from liver.
- About 95% of vitamin A is stored as its palmitate ester in the liver. It is released in plasma as and when required.

Q-5 What are dietary source and dietary requirement of Vitamin A?

A-5 High content of vit.A - 10,000 to 76.000 I.U. /100 gm. i.e. Liver - beef, pig, sheep, chicken & calf, Liver oil - cod, shark, whale, salmon, Plants - carrots, spinach, mint, turnip green, parselyand palm oil.

Medium content of vit-A - 1000 to 10,000 I.U./100gm. i.e. Butter, cheese, egg yolk, dried milk, cream. Plants - mangoes, cherries, peaches, pumpkin, tomatoes, lettuce, sweet potatoes, apricots, papaya.

Low content of vit.A - 100 to 1,000 I. U. /100gm. Milk, Grapes, banana, berries, olives, oranges, pineapples, prunes, avocadoes Okra, peanuts, pecan.

Units; 1 I.U.= 0.3μg retinal = 0.344μg. vit. A acetate. 1RE (retinal equivalent)=1 mg. retinol = 6mg. β carotene =12 mg. other carotenes.

Daily requirement: Children ------- 2000 ---3,500 I.U./day
Adult---------- 5,000 I.U./day.
Pregnancy------- 6,000 I.U./day.
Lactation---------8,000 I.U./day.

Q-6 Describe the role of vitamin A in vision:

A-6

- The molecular mechanism through which vitamin A functions in visual system is known as Rhodopsin cycle or Wald's visual cycle for which George Wald was awarded Nobel Prize.
- Photoreception in the eye is the function of two specialized cell types located in the retina; the rod and cone cells. Rod cells are sensitive and function in dim illumination, are concentrated around the periphery of the retina are about 100 million cells.
- Both rod and cone cells contain a photoreceptor pigment in their membranes.
- The photosensitive compound of most mammalian eyes is a protein called opsin to which is covalently coupled an aldehyde of vitamin A, and is called rhodopsin or visual purple.
- This compound is a complex between opsin and the 11-*cis*-retinal (also called 11-*cis*-retinene) form of vitamin A.

- It is sensitive to light and when illuminated, it changes from red to orange yellow, and on prolonged exposure, to colorless retinal and opsin.
- This process triggers a nerve impulse that is transmitted by the optic nerve to brain.
- On exposure to light this gets bleached and is converted to all-trans-retinal (visual yellow) and opsin.
- In the dark rhodopsin is regenerated and sensitivity of the retina is restored. (Dark adaptation)
- Light, especially short wavelengths, isomerizes trans retinine to the cis form. This reaction is slow.
- An enzyme in the retina specially and rapidly (especially in dim light) catalyzes the conversion of all trans retinene to 11-cis retinene (neo-b retinine), by the enzyme retinene isomerase.
- 11-cis-retinal then combines spontaneously with opsin in the dark to form rhodopsin, thus completing the cycle.
- It is important to note that all-trans-retinal is incompletely converted to 11-cis-retinal; hence a constant supply of vitamin A is needed in the diet.

Q-7 **Describe other functions of vitamin A, besides visual cycle.**

A-7

Growth : Vitamin A helps in the formation of chondritin sulfate in cartilage. In absence of Vitamin A, animal fails to grow, bone growth is slow.

Anemia: Vitamin A deficiency can lead to anemia caused by impaired mobilization of iron from the liver because vitamin A is required for the synthesis of iron transport protein transferin.

Bone and Teeth: Defective formation of enamel of teeth. Abnormal bone and tooth formation are reversed by vitamin A administration.

Carbohydrate metabolism: Vitamin A helps glucose synthesis from triose molecules. **Reproduction:** Retinol and retinal are essential for normal reproduction.

Urolithiasis: In vitamin A deficiency, urino-genital epithelium shows keratinization followed by bacterial invasion and formation of alkaline urine. This factors cause calcium phosphate precipitation, which leads to formation of urinary calculi.

Xeropthalmia: Xeropthalmia is an eye disease characterized by drying of the eyes. The cells of lachrymal glands become keratinized and stops secreting tears hence the bacteria are not washed away. The external surface of cornea becomes dry with dull appearance. The eyelids, swells and becomes sticky and there will be severe eye infection. Ulcers may develop and if not treated in time blindness results. Less severe sub clinical symptoms frequently found in human beings is night blindness or nyctalopia, inability to see in dim light or to adopt to decrease intensity of light.

Cancer: The increased risk of cancer in vitamin deficiency is thought to be the result of depletion in beta-carotene. Beta-carotene is a very effective antioxidant and is suspected to reduce the risk of cancer is known to be initiated, by the production of free radicals. Of particular interest is the potential benefit of increased b-carotene intake to reduce the risk of lung cancer in smokers. However, caution needs to be taken when increasing the intake of any of the lipid soluble vitamins.

Q-8 List Vitamin A Toxicity manifestations.

A-8

- Fatigue insomnia, irritability, nausea, diarrhea are observed
- Nerve lesion, painful bone & joints
- Abnormal bone growth
- Loss of hair · Liver is enlarged and becomes cirrhotic
- Decrease in blood clotting time.

Q-9 Vitamin D is structurally related to which compound?

A-9 Vitamin D is a fat-soluble vitamin (acts as a steroid hormone) and is structurally related to a group of steroids that occur mainly in animals (cholecalciferol) but also in plants and yeasts (ergosterol).

Q-10 How cholesterol is structurally related to vitamin D?

A-10 Provitamin D is a simple derivative of cholesterol, which occurs when a hydrogen is removed from the number 7 carbon, which then forms a double bond with the number 8 carbon, in the second, or 'B' ring of the cholesterol molecule.

The cholesterol is 'oxidized' (that is, an electron is removed with the hydrogen atom), so that the double bond is a consequence of 2 mutually shared electrons between carbons 7 and 8.

Q-11 What is the structural difference between pro-vitamin D (7-dehydro cholesterol) in the skin and ergosterol?

A-11 Ergosterol and pro-vitamin D, 7-dehydrocholesterol, in the skin have the same structure except ergosterol has one more double bond in the side chain between C22 and C23 and has one more methyl group at C24. Both the provitamin D and ergosterol are converted to active vitamin D by UV radiation.

Q-12 How vitamin D2 and D3 are named as?

A-12 Vitamin D2 is of vegetable origin called as Ergocalciferol.

Vitamin D3 is of animal origin known as cholecalciferol.

Q-12 How vitamin D is absorbed and transported?

A-12 Cholecalciferol (or Ergocalciferol) is absorbed from the intestinal tract and requires the presence of bile salts, after absorption it is transported to the liver bound to a specific vitamin D-binding protein (alpha-2 globulin).

Q-13 How Vitamin D is activated to its biologically active form?

A-13

- After absorption vitamin D2 and D3 are transported to liver bound to alpha-2 globulin but are not biologically active.
- They are converted to active form by two sequential hydroxylation reactions.
- The first hydroxylation occurs at the 25-position and is catalyzed by specific mitochondrial hydroxylase in the liver.
- The product of the reaction 25-hydroxycholecalciferol or 25 -OH D3 is the predominant form of vitamin D in the plasma and is the major form in which vitamin D is stored.
- Liver 25-hydroxylase does not have any specific regulatory mechanism and hence the 25-hydroxy D level is dependent upon vitamin D intake and the extent to which skin is exposed to sunlight.
- 25-OH D_3 is further hydroxylated at 1-position by a specific 25-OH-cholecalciferol 1-hydroxylase enzyme, primarily present in the proximal convoluted tubules of kidney, forming 1,25-dihydroxycholecalciferol (DHCC) with the help of cytochromP-450, molecular oxygen & NADPH.
- 1,25-dihydroxycholecalciferol, or active Vitamin D, contains three hydroxyl groups at 1, 3 and 25 positions. So it is called calcitriol and it is biologically active form of vitamin D.
- Vitamin D_2 and D_3 are processed to D_2-calcitriol and D_3-calcitriol, respectively by the same enzymatic pathways in the body.
- This 1-hydroxylase enzyme is controlled by parathyroid hormone (PTH), and by calcium and phosphate concentrations in the blood, as well as by feedback regulation from the active form of the vitamin.
- Calcitriol (1,25-dihydroxycholecalciferol) is the biologically active form of Vitamin D, and it is transported in the bloodstream to its major sites of action: intestine, kidney and bone tissue.

Q-14 What is the mode of action of Vitamin D?

A-14

- Vitamin D is a steroid hormone that functions to regulate specific gene expression following interaction with its intracellular receptor.
- Calcitriol binds to a cytoplasmic receptor.

- The hormone-receptor complex interacts with DNA and causes transcription of specific genes that code for a specific calcium binding protein, calbindin.
- Due to the increased availability of calcium binding protein, the absorption of calcium is increased.
- The increased absorption of calcium ions requires concomitant absorption of a negatively charged counter ion to maintain electrical neutrality; the predominant counter ion is phosphate.
- Calcitriol functions in concert with parathyroid hormone (PTH) and calcitonin to regulate serum calcium and phosphorous levels.

Q-15 Discuss functions of vitamin D.

A-15

- Increases protein synthesis in intestinal cells.
- Activates active transport of calcium by intestinal cells.
- Increases release of calcium by mitochondria.
- Promotes normal bone calcification.
- Regulates phosphorus and calcium metabolism.
- Increases renal tubular reabsorption of calcium.
- It does not have any coenzyme activity.

Q-16 Discuss the role of vitamin D in regulating calcium and phosphorus levels in body.

A-16

- Active calcitriol stimulates production of calcium binding protein in the mucosal cells of intestine, which helps absorption of more calcium.
- The two primary stimuli for increasing activity of 1-hydroxylase in the kidney are low levels of calcium and phosphorus.
- Low serum phosphate level directly stimulates the enzyme 1-hydroxylase.
- Low serum calcium level indirectly stimulates the enzyme 1-hydroxylase, via PTH.
- Low serum calcium ion stimulates parathyroid glands to secrete PTH, which in turn stimulates 1-hydroxylase and inhibits 24-hydroxylase reactions in kidney.
- PTH is released in response to low serum calcium and induces the production of calcitriol. In contrast, reduced levels of PTH stimulate synthesis of the inactive $24,25\text{-}(OH)_2D_3$.
- When plasma calcium level falls the major sites of action of calcitriol and PTH are bone where they stimulate bone resorption and the kidneys where they inhibit calcium excretion by stimulating reabsorption by the distal tubules.

- At the same time it prevents reabsorption of phosphate by kidney and phosphate is excreted in the urine.
- Feed back inhibition of 1-hydroxylase occurs, once the hypocalcaemia and hypophosphatemia is corrected.
- Corticosteroids stimulate the conversion of vitamin D to its inactive metabolite and have been shown to cause bone demineralization when used for long period of time.

Q-17 How is Vitamin D deficiency manifested? Or Clinical Significance of Vitamin D Deficiency.

A-17

- As a result of the addition of vitamin D to milk, deficiencies in this vitamin are rare.
- The main symptom of vitamin D deficiency in children is rickets and in adults is osteomalacia.
- Rickets is characterized improper mineralization during the development of the bones resulting in soft, pliable bones.
- Renal rickets (renal osteodystrophy) results from chronic renal failure where decreased ability to from active from of vitamin D.
- In adults, osteomalacia is a condition of defective new bone mineralization in the adult skeleton due to Vitamin D deficiency.
- Osteomalacia is characterized by demineralization of previously formed bone leading to increased softness and susceptibility to fracture.

Q-18 Describe sources and daily requirement of vitamin D.

A-18

- Good sources: Fish liver oil (Tuna, Cod, Halibut and shark).
- Medium sources: Egg yolk, margarine, lard, shrimps and sardine.
- Low sources: grain and vegetable oils, butter, cream, cheese, milk, meat, beef.
- The cheapest source is sunlight, which converts 7-dehydrocholesterol to vitamin D in the skin.
- The daily requirement of an adult is 200 IU and that of children and women during pregnancy and lactation is 400 IU. One IU= 0.025 mcg of vitamin D3.

Q-19 Write a short note on hypervitaminosis D.

A-19 Vitamin D is most toxic of all vitamins stored in the body. It is slowly metabolized. Extremely large amounts (500-1000times RD) cause hypervitaminosis D. The clinical manifestations are:

- Anorexia, thirst, constipation and polyuria.
- This is followed by nausea, vomiting and diarrhea.
- Hyperphosphatemia also occurs.

- Hypercalcaemia and hyperphosphatemia may lead to metastatic calcification. The kidney, arteries, muscles and gastric mucosa are mainly involved.
- Increased urinary excretion of calcium and phosphate may lead to urinary lithiasis.
- The renal failure leads to death.

Q-20 What is Vitamin E? Discuss its role in the body.

A-20 Vitamin E is a mixture of several related compounds known as tocopherols.

- The α-tocopherol molecule is the most potent of the tocopherols. The active form of Vitamin E is alpha-tocopherol.
- Vitamin E is absorbed from the intestines packaged in chylomicrons. It is delivered to the tissues via chylomicron transport and then to the liver through chylomicron remnant uptake. The liver can export vitamin E in VLDLs.
- Due to its lipophilic nature, vitamin E accumulates in cellular membranes, fat deposits and other circulating lipoproteins.
- The major site of vitamin E storage is in adipose tissue.
- Vitamin E is necessary for normal reproduction, muscular development and resistance of erythrocytes to hemolysis.
- The major function of vitamin E is to act as a natural antioxidant by scavenging free radicals and molecular oxygen. In particular vitamin E is important for preventing peroxidation of polyunsaturated membrane fatty acids.
- The vitamins E and C are interrelated in their antioxidant capabilities.
- Active a-tocopherol can be regenerated by interaction with vitamin C following scavenge of a peroxy free radical. Alternatively, a-tocopherol can scavenge two peroxy free radicals and then be conjugated to glucuronate for excretion in the bile.
- It acts synergistically with selenium as an anti-oxidant.

Q-21 Describe clinical manifestations of Vitamin E deficiency.

A-21

- No major disease states have been found to be associated with vitamin E deficiency due to adequate levels in the average diet.
- The major symptom of vitamin E deficiency in humans is an increase in red blood cell fragility.
- Since vitamin E is absorbed from the intestines in chylomicrons, any fat malabsorption diseases can lead to deficiencies in vitamin E intake.
- Neurological disorders have been associated with vitamin E deficiencies associated with fat malabsorptive disorders.

- Increased intake of vitamin E is recommended in premature infants fed formulas that are low in the vitamin as well as in persons consuming a diet high in polyunsaturated fatty acids.
- Polyunsaturated fatty acids tend to form free radicals upon exposure to oxygen and this may lead to an increased risk of certain cancers.
- Deficiency can also lead to degeneration of the central nervous system.

Q-22 What are the dietary sources and daily requirement of Vitamin E?

A-22

- Vegetable oils are rich sources of Vitamin E, e.g. wheat germ oil, sunflower oil, safflower oil, cottonseed oil, and palm oil.
- Sources of Vitamin E are found in vegetables, fruits, liver & eggs. Over 60% of dietary Vitamin E comes from vegetable oil.
- Daily requirement of vitamin E is about 15 mg or 33 international units.
- One mg of d-α-tocopherol acetate is equal to one IU.

Q-23 Describe briefly the structure of Vitamin K and its biochemical role.

A-23

- The K vitamins exist naturally as K_1 (phytylmenaquinone) in green vegetables and K_2 (multiprenylmenaquinone) in intestinal bacteria and K_3 is synthetic menadione. They are naphthoquinone derivatives.
- Vitamin K is a fat-soluble vitamin, which is stored in the liver. It plays a role in the complicated process of blood clotting.
- The major function of the K vitamins is in the maintenance of normal levels of the blood clotting proteins, factors II, VII, IX, X and protein C and protein S, which are synthesized in the liver as inactive precursor proteins.
- Conversion from inactive to active clotting factor requires a post-translational modification of specific glutamate (E) residues. This modification is a carboxylation and the enzyme responsible for it requires vitamin K as a cofactor.
- The resultant modified E residues are gamma-carboxyglutamate (*gla*). This process is most clearly understood for factor II, also called pre-prothrombin. Prothrombin is modified pre-prothrombin. The *gla* residues are effective calcium ion chelators. Upon chelation of calcium, prothrombin interacts with phospholipids in membranes and is proteolysed to thrombin through the action of activated factor X (Xa).
- During the carboxylation reaction reduced hydroquinone form of vitamin K is converted to a 2,3-epoxide form. The regeneration of the hydroquinone form requires an uncharacterized reductase. This latter reaction is the site of action of the dicumarol-based anticoagulants such as warfarin.
- Dicumarol competitively inhibits vitamin K epoxide reductase.

Q-24 Discuss clinical significance of Vitamin K Deficiency.

A-24

- Naturally occurring vitamin K is absorbed from the intestines only in the presence of bile salts and other lipids through interaction with chylomicrons. Therefore, fat malabsorptive diseases can result in vitamin K deficiency.
- The synthetic vitamin K_3 is water soluble and absorbed irrespective of the presence of intestinal lipids and bile. Since the vitamin K_2 form is synthesized by intestinal bacteria, deficiency of the vitamin in adults is rare.
- However, long-term antibiotic treatment can lead to deficiency in adults.
- The intestine of newborn infants is sterile, therefore, vitamin K deficiency in infants is possible if lacking from the early diet.
- The primary symptom of a deficiency in infants is a hemorrhagic syndrome.
- Obstructive jaundice and antibiotic therapy will result in vitamin K deficiency.
- Administration of dicumarol (to prevent intravascular thrombosis) will result in vitamin K deficiency.
- Deficiency in Vitamin K promotes delays in clotting. Excessive bleeding and bruises under the skin are symptoms.
- Q19. What are the dietary sources and daily requirement of Vitamin K?
- A19.
- Vitamin K is obtained from food sources such as alfalfa (also containing vitamin E which promotes absorption of vitamin K), kelp, all green leafy vegetables, tomatoes, cabbage, spinach, whole grain cereals and milk.
- Vitamin K is also produced within the body by the bacterial flora in the large intestine and it is thought that about 50% of the vitamin K in the blood of an adult is of bacterial origin.
- In the newborn however, the gut is sterile and it takes 5 to 7 days for it to become colonised by bacteria.

Water - soluble vitamins

Q-25 How chemical structures of water-soluble vitamins are related to their functions?

A-25 In general, the water soluble vitamins consists of:

- Derivatives or substituted derivatives of sugars (vit-C),
- Derivatives of pyridine (niacin, B6),
- Derivatives of purines and pyrimidines (folic acid, B2, B1),
- Amino acid-organic acid complex (folic acid, biotin, pantothenic acid) and
- A porphyrin-nucleotide complex (B12).

These structurally diverse water-soluble vitamins act:

- As enzyme activators and coenzymes (B1, B2, B6, B12, pantothenic acid, folic acid, biotin, niacin) o As redox agent on enzyme reactions (Vit-C, B2, B12, folic acid, niacin)
- ✓ As nuclear agent (folic acid, B12, Vit-C, biotin)
- ✓ As mitochondrial agent (B2, Vit-C, niacin)

Q-26 Describe briefly the structure of thiamine and its active form.

A-26

- Thiamine is also known as vitamin B1.
- Thiamin is derived from a substituted pyrimidine and a thiazole, which are coupled by a methylene bridge.
- Thiamin is rapidly converted to its active form, thiamin pyrophosphate, TPP, in the brain and liver by a specific enzyme, *thiamin diphosphotransferase*.
- TPP is necessary as a cofactor for the *pyruvate* and *a-ketoglutarate dehydrogenase* catalyzed reactions as well as the *transketolase* catalyzed reactions of the pentose phosphate pathway.

Q-27 What is dietary requirement of Thiamine (Vitamin B1)?

A-27 The dietary requirement for thiamine is proportional to the caloric intake of the diet and ranges from 1.0 - 1.5 mg/day for normal adults. If the carbohydrate content of the diet is excessive then an increased thiamine intake will be required. Requirement is increased in pregnancy and lactation. It also depends of intestinal synthesis and absorption and fat content of diet (increased Pyruvate).

Q-28 What are dietary sources of Vitamin B1?

A-28 Following are the dietary sources of Vitamin B1:

High: 1000-10,000microgram/100g, Wheat germ, rice bran, soybean flour yeast and ham.

Medium: 100-1000microgram/100g, Peanuts, pecan, walnut, almonds, sprouts, broccoli, cauliflower, potatoes, beans, eggs, milk and beef whole grain cereals and breads.

Low: 10-100microgram/100g, Apples, berries, banana, oranges, dates, beet, cabbage, carrot, radish, spinach etc.

Q-29 Describe biochemical role of thiamine.

A-29

- Active form of thiamine, that is thiamine pyrophosphate (TPP) is required for the key reactions catalyzed by Pyruvate dehydrogenase complex and alpha-ketoglutarate dehydrogenase complex (TCA cycle).
- Thiamine deficiency results in inhibition of carbohydrate metabolism causing accumulation of Pyruvate.

- TPP is also required for transketolase reaction of the pentose phosphate pathway.
- TPP functions in transmission of nerve impulses and is localized in peripheral nerve membranes.
- It is required for acetylcholine synthesis.
- It is required for ion translocation reactions in stimulated neural tissue.

Q-30 Describe clinical manifestations of thiamine deficiency.

A-30

- Vitamin B1 is necessary to breakdown & release energy from carbohydrates. It is also necessary for the structure of the nerve membranes.
- A deficiency in thiamin intake leads to a severely reduced capacity of cells to generate energy as a result of its role in these reactions.
- The earliest symptoms of thiamin deficiency include constipation, loss of appetite, nausea as well as mental depression, peripheral neuropathy and fatigue.
- Chronic thiamin deficiency leads to more severe neurological symptoms including ataxia, mental confusion and loss of eye coordination.
- Other clinical symptoms of prolonged thiamin deficiency are related to cardiovascular and musculature defects.
- The severe deficiency of thiamine produces the disease called Beriberi, which affects the brain, heart, and nerves. This disease is prevalent in the Orient because of the abundance of rice they consume. The rice has been milled which strips the rice of thiamine.
- An alcohol-related thiamine deficiency, *Wernicke-Korsakoff Syndrome*, is caused by inadequate intake of thiamine as well as impaired absorption and storage, and is the common cause of dementia.
- Branched-chain ketoaciduria (commonly known as Maple Syrup Urine Disease; MSUD) is another ailment that may be caused by thiamine deficiency. In MSUD, the oxidative decarboxylation of alpha-keto acids derived from, i.e. valine, isoleucine, and leucine, is blocked due to an inadequate supply of the coenzyme thiamine pyrophosphate (TPP). Clinical symptoms of MSUD include mental and physical retardation.

Q-31 Describe briefly the structure of Riboflavin (Vitamin B-2) and its biochemical role.

A-31

- Riboflavin is the precursor for the coenzymes, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD).

- The enzymes that require FMN or FAD as cofactors are termed flavoproteins. Several flavoproteins also contain metal ions and are termed metalloflavoproteins.
- Both classes of enzymes are involved in a wide range of redox reactions, e.g. *succinate dehydrogenase* and *xanthine oxidase.*
- During the course of the enzymatic reactions involving the flavoproteins the reduced forms of FMN and FAD are formed, $FMNH_2$ and $FADH_2$, respectively.
- The flavin coenzymes are essential for energy production and cellular respiration.

Q-32 What is dietary requirement of Riboflavin (Vitamin B-2) and what are dietary sources of it?

A-32 The normal daily requirement for riboflavin is 1.2 - 1.7 mg/day for normal adults.

Following are the dietary sources of Vitamin B-2:

High: 1000-10,000microgram/100g, Beef, chicken, pork, yeast.

Medium: 100-1000microgram/100g, Avocados, currents, asparagus, beans, sprouts, egg, milk, nuts.

Low: 10-100microgram/100g, Apples, banana, oranges, dates, carrot, rice

Q-33 Describe clinical manifestations of riboflavin deficiency.

A-33 Symptoms associated with riboflavin deficiency include:

- Inflammation or open sores at the corners of the mouth or lips, a purple - red inflamed tongue, angular stomatitis, glossitis, cheilosis, photophobia & seborrheic dermatitis (dandruff).
- Riboflavin decomposes when exposed to visible light. This characteristic can lead to riboflavin deficiencies in newborns treated for hyperbilirubinemia by phototherapy.
- Riboflavin deficiency is often seen in chronic alcoholics due to their poor dietetic habits.

Q-34 Describe briefly the structure of Niacin and its biochemical role.

A-34

- Niacin (nicotinic acid and nicotinamide) is also known as vitamin B_3. Both nicotinic acid and nicotinamide can serve as the dietary source of vitamin B_3.
- Niacin is required for the synthesis of the active forms of vitamin B_3, nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$).
- Both NAD^+ and $NADP^+$ function as cofactors for numerous dehydrogenases and oxidases class of enzymes.

- In anabolic role it maintains microsomal reductive biosynthesis and photolsynthesis.
- In catabolic role furnishes coenzymes for lipid catabolism, oxidative deamination and key reactions in TCA cycle.
- It acts as hydrogen and electron transfer agent and maintains respiratory chain in mitochondria.
- Niacin is not a true vitamin in the strictest definition since it can be derived from the amino acid tryptophan. However, the ability to utilize tryptophan for niacin synthesis is inefficient (60 mg of tryptophan are required to synthesize 1 mg of niacin). Also, synthesis of niacin from tryptophan requires vitamins B_1, B_2 and B_6, which would be limiting in them on a marginal diet.

Q-35 What is dietary requirement of Niacin and what are dietary sources of it?

A-35 The recommended daily requirement for niacin is 13 - 19 niacin equivalents (NE) per day for a normal adult. One NE is equivalent to 1 mg of free niacin).

Following are the dietary sources of Vitamin B-2:

High: 10-100mg/100g, Peanut, rice bran, liver, heart, Beef, chicken, tuna, yeast.

Medium: 1-10mg/100g, Avocados, dates, figs, beans, sprouts, nuts.

Low: 0.1-1.0mg/100g, Apples, banana, berries, melon, peach, oranges, sprouts, tomato

Q-36 Describe clinical manifestations of Niacin deficiency.

A-36

- A diet deficient in niacin (as well as tryptophan) leads to glossitis of the tongue, dermatitis, weight loss, diarrhea, depression and dementia.
- Deficiency in niacin causes *pellagra* (rough skin). Pellagra involves the skin and digestive and nervous system. Symptoms are the 4 D's: Dermatitis, Diarrhea, Dementia, & Death. Niacin also has vasodilating activity.
- Several physiological conditions (e.g. Hartnup disease and malignant carcinoid syndrome) can lead to niacin deficiency.
- In Hartnup disease tryptophan absorption is impaired and in malignant carcinoid syndrome tryptophan metabolism is altered resulting in excess serotonin synthesis.
- Certain drug therapies (e.g. isoniazid) can lead to niacin deficiency. Isoniazid (the hydrazide derivative of isonicotinic acid) is the primary drug for chemotherapy of tuberculosis.
- Nicotinic acid (but not nicotinamide) when administered in pharmacological doses of 2 - 4 g/day lowers plasma cholesterol levels and has been shown to be a useful therapeutic for hypercholesterolemia. The

major action of nicotinic acid in this capacity is a reduction in fatty acid mobilization from adipose tissue. Although nicotinic acid therapy lowers blood cholesterol it also causes a depletion of glycogen stores and fat reserves in skeletal and cardiac muscle. Additionally, there is an elevation in blood glucose and uric acid production. For these reasons nicotinic acid therapy is not recommended for diabetics or persons who suffer from gout.

Q-37 Describe briefly the structure of Vitamin B-6 and its biochemical role.

A-37

- Pyridoxal, pyridoxamine and pyridoxine are collectively known as vitamin-B6. All three compounds are efficiently converted to the biologically active form of vitamin-B6, pyridoxal phosphate. The ATP requiring enzyme, pyridoxal kinase, catalyzes this conversion.
- Vitamin B6 is a component of a coenzyme.
- Pyridoxal phosphate is essential for energy production from amino acids and can be considered an energy-releasing vitamin.
- It is active in converting important amino acids to ketoacids.
- Pyridoxal phosphate functions as a cofactor in enzymes involved in transamination reactions required for the synthesis and catabolism of the amino acids.
- It acts as a cofactor for *glycogen phosphorylase* in glycogenolysis. Decreased glucose tolerance may be associated with vitamin B-6 deficiency.
- Pyridoxl phosphate is required for synthesis of neurotransmitters serotonin and norepinephrine.
- It is necessary for the synthesis of sphingolipids necessary for myelin formation. This explains the irritability, nervousness and depression found with mild deficiency and peripheral neuropathy and convulsions observed with severe deficiency.
- Pyridoxal phosphate is required for the synthesis of delta aminolevulinic acid, a precursor of heme. Vitamin B-6 deficiency occasionally cause sideroblastic anemia, which is characteristically a microcytic anemia, observed in the presence of high serum iron.
- Vitamin B-6 is also required for the conversion of homocysteine to cysteine and hyperhomocysteinemia appears to be a risk factor for cardiovascular disease.
- Pyridoxal phosphate is one of the cofactor required for conversion of tryptophan to NAD.

Q-38 What is dietary requirement of Vitamin B-6 and what are dietary sources of it?

A-38 The requirement for vitamin B_6 in the diet is proportional to the level of protein consumption ranging from 1.4 - 2.0 mg/day for a normal adult.

Following are the dietary sources of Vitamin B-6:

High: 1000-10,000mcg/100g, Walnut, peanut, wheat germ, brown rice, yeast, liver (Beef), herring and Salmon.

Medium: 100-1000mcg/100g, Banana, Avocados, grapes, pears. Cabbage, carrots, peas, potatoes, tomatoes, spinach, soybean, wheat, butter and eggs.

Low: 10-100mcg/100g, Apples, oranges, raisins, watermelon, asparagus, bens, lettuce, onion, cheese and milk.

Q-39 Describe clinical manifestations of Vitamin B-6 deficiency.

A-39 Deficiency of Vitamin B6 can cause convulsions, lethargy, mental changes & retardation, anemia, and skin inflammation. Deficiencies of vitamin B_6 are rare and usually are related to an overall deficiency of all the B-complex vitamins. Isoniazid (see niacin deficiencies above) and penicillamine (used to treat rheumatoid arthritis and cystinurias) are two drugs that complex with pyridoxal and pyridoxal phosphate resulting in a deficiency in this vitamin.

Q-40 Write a short note on Pantohenic acid.

A-40

- Pantothenic acid is also known as vitamin B_5.
- Pantothenic acid is formed from b-alanine and pantoic acid.
- Pantothenic acid is required for synthesis of coenzyme A, CoA and is a component of the acyl carrier protein (ACP) domain of fatty acid synthase.
- Pantothenic acid is, therefore, required for the metabolism of carbohydrate via the TCA cycle and all fats and proteins.
- At least 70 enzymes have been identified as requiring CoA or ACP derivatives for their function.
- Deficiency of pantothenic acid is extremely rare due to its widespread distribution in whole grain cereals, legumes and meat.
- Symptoms of pantothenate deficiency are difficult to assess since they are subtle and resemble those of other B vitamin deficiencies.
- Present in all living tissues almost entirely in the form of a coenzyme A (CoA). This coenzyme has many metabolic roles in the cell and lack of pantothenic acid can lead to depressed metabolism of both carbohydrates and fats.

Q-41 Write a short note on Biotin.

A-41 Biotin is the prosthetic group for number of caboxylation reactions e.g.

- Pyruvate carboxylase (for synthesis of oxaloacetate for gluconeogensis and replenishment of citric acid cycle.
- Acetyl-CoA carboxylase (fatty acid biosynthesis) and
- Propionyl-CoA carboxlase (methionine, leucine and valine metabolism)

Biotin is found in numerous foods and also is synthesized by intestinal bacteria and as such deficiencies of the vitamin are rare.

Deficiencies are generally seen only after long antibiotic therapies, which deplete the intestinal fauna or following excessive consumption of raw eggs. The latter is due to the affinity of the egg white protein, avidin, for biotin preventing intestinal absorption of the biotin.

Q-42 Describe briefly the structure of Vitamin B-12 and its biochemical role.

A-42

- Vitamin B_{12} is composed of a complex tetrapyrrol ring structure (corrin ring) and a cobalt ion in the center. It is also known as cobalamin.
- Vitamin B_{12} is synthesized exclusively by microorganisms and is found in the liver of animals bound to protein as methycobalamin or 5'-deoxyadenosylcobalamin.
- The vitamin must be hydrolyzed from protein in order to be active. Hydrolysis occurs in the stomach by gastric acids or the intestines by trypsin digestion following consumption of animal meat.
- The vitamin is then bound by intrinsic factor, a protein secreted by parietal cells of the stomach, and carried to the ileum where it is absorbed.
- Following absorption the vitamin is transported to the liver in the blood bound to transcobalamin II.

There are only two clinically significant reactions in the body that require vitamin B_{12} as a cofactor.

1. During the catabolism of fatty acids with an odd number of carbon atoms and the amino acids valine, isoleucine and threonine the resultant propionyl-CoA is converted to succinyl-CoA for oxidation in the TCA cycle. One of the enzymes in this pathway, *methylmalonyl-CoA mutase,* requires vitamin B_{12} as a cofactor in the conversion of methylmalonyl-CoA to succinyl-CoA. The 5'-deoxyadenosine derivative of cobalamin is required for this reaction.
2. The second reaction requiring vitamin B_{12} catalyzes the conversion of homocysteine to methionine and is catalyzed by *methionine synthase.* This reaction results in the transfer of the methyl group from N^5-methyltetrahydrofolate to hydroxycobalamin generating tetrahydrofolate and methylcobalamin during the process of the conversion.

Q-43 Describe source, requirement and deficiency manifestations of Vitamin B-12.

A-43 Vitamin B12 is not found in plant foods. The main source of B12 in human diet is through animal products like milk, eggs and liver. Vitamin B12 requires the presence of intrinsic factor from the stomach in order to be absorbed in the small intestines. The liver can store up to six years worth of vitamin B-12, hence deficiencies in this vitamin are rare.

B12 is needed for the efficient production of blood cells and for the health of the nervous system.

The inability to absorb Vitamin B12 occurs in *pernicious anemia.* In pernicious anemia intrinsic factor is missing. The anemia results from impaired DNA synthesis due to a block in purine and thymidine biosynthesis. The block in nucleotide biosynthesis is a consequence of the effect of vitamin B_{12} on folate metabolism. When vitamin B-12 is deficient essentially all of the folate becomes trapped as the N^5-methyltetrahydrofolate derivative as a result of the loss of functional methionine synthase. This trapping prevents the synthesis of other tetrahydrofolate derivatives required for the purine and thymidine nucleotide biosynthesis pathways.

Neurological complications also are associated with vitamin B-12 deficiency and result from a progressive demyelination of nerve cells. The demyelination is thought to result from the increase in methylmalonyl-CoA that result from vitamin B-12 deficiency. Methylmalonyl-CoA is a competitive inhibitor of malonyl-CoA in fatty acid biosynthesis as well as being able to substitute for malonyl-CoA in any fatty acid biosynthesis that may occur. Since the myelin sheath is in continual flux the methylmalonyl-CoA-induced inhibition of fatty acid synthesis results in the eventual destruction of the sheath. The incorporation methylmalonyl-CoA into fatty acid biosynthesis results in branched-chain fatty acids being produced that may severely alter the architecture of the normal membrane structure of nerve cells

Q-44 Write short not on Folic acid and its biochemical role.

A-44 The active form of folic acid is folacin.

- Folic acid is a conjugated molecule consisting of a pteridine ring structure linked to para-aminobenzoic acid (PABA) that forms pteroic acid.
- Folic acid itself is then generated through the conjugation of glutamic acid residues to pteroic acid.
- When stored in the liver or ingested folic acid exists in a polyglutamate form. Intestinal mucosal cells remove some of the glutamate residues through the action of the lysosomal enzyme, conjugase. The removal of glutamate residues makes folate less negatively charged (from the

polyglutamic acids) and therefore more capable of passing through the basal lamenal membrane of the epithelial cells of the intestine and into the bloodstream.

- Folic acid is reduced within cells (principally the liver where it is stored) to tetrahydrofolate (THF also H_4folate) through the action of dihydrofolate reductase (DHFR), an NADPH-requiring enzyme.

  The function of THF derivatives is to carry and transfer various forms of one-carbon units during biosynthetic reactions.

  The one-carbon units are methyl, methylene, methenyl, formyl or formimino groups. These one-carbon transfer reactions are required in the biosynthesis of serine, methionine, glycine, choline and the purine nucleotides and dTMP.

  The ability to acquire choline and amino acids from the diet and to salvage the purine nucleotides makes the role of N^5, N10-methylene-THF in dTMP synthesis the most metabolically significant function for this vitamin.

  The role of vitamin B_{12} and N^5-methyl-THF in the conversion of homocysteine to methionine also can have a significant impact on the ability of cells to regenerate needed THF.

Q-45 Describe source, requirement and deficiency manifestations of Folic acid.

A-45

- Folic acid is obtained primarily from yeasts and leafy vegetables as well as animal liver. Animal cannot synthesize PABA nor attach glutamate residues to pteroic acid, thus, requiring folate intake in the diet.
- The body needs folic acid and folates (form of folic acid that occurs in food) to make DNA. Rapidly dividing cells in the blood, the lining of the colon and developing neutral tube need folic acid the most.
- Folic acid can prevent at least some children from being born with spina bifida or other birth defects.
- Folic acid might prevent heart disease in adults by lowering levels of an artery damaging substance called homocysteine. Homocysteine is an amino acid that's used to make protein. It could damage arteries. Folic acid along with Vitamin B12 &B6 all are needed to convert homocysteine to other things.
- Folic acid is also used in the treatment of *sprue*- a chronic form of malabsorption.
- Folate deficiency results in complications nearly identical to those described for vitamin B-12 deficiency.
- The most pronounced effect of folate deficiency on cellular processes is upon DNA synthesis. This is due to impairment in dTMP synthesis, which

leads to cell cycle arrest in S-phase of rapidly proliferating cells, in particular hematopoietic cells. The result is during erythrocyte maturation leads to abnormally large erythrocytes termed macrocytic anemia.

- Folate deficiencies are rare due to the adequate presence of folate in food. Poor dietary habits as those of chronic alcoholics can lead to folate deficiency.
- The predominant causes of folate deficiency in non-alcoholics are impaired absorption or metabolism or an increased demand for the vitamin.
  The predominant condition requiring an increase in the daily intake of folate is pregnancy. This is due to an increased number of rapidly proliferating cells present in the blood.
- The need for folate will nearly double by the third trimester of pregnancy.
- Certain drugs such as anticonvulsants and oral contraceptives can impair the absorption of folate. Anticonvulsants also increase the rate of folate metabolism.

Q-46 Write a short note on Vitamin C.

A-46 Vitamin C is also known as Ascorbic acid.

- Ascorbic acid is derived from glucose via the uronic acid pathway. The enzyme L-gulonolactone oxidase responsible for the conversion of gulonolactone to ascorbic acid is absent in primates, thus making ascorbic acid an essential in the diet.
- The active form of vitamin C is ascorbate acid itself.
- The main function of ascorbate is as a reducing agent in a number of different reactions. Vitamin C has the potential to reduce cytochromes a and c of the respiratory chain as well as molecular oxygen.
- The most important reaction requiring ascorbate as a cofactor is the hydroxylation of proline residues in collagen. Vitamin C is, therefore, required for the maintenance of normal connective tissue as well as for wound healing since synthesis of connective tissue is the first event in wound tissue remodeling.
- Vitamin C also is necessary for bone remodeling due to the presence of collagen in the organic matrix of bones.
- Several other metabolic reactions require vitamin C as a cofactor. These include the catabolism of tyrosine and the synthesis of epinephrine from tyrosine and the synthesis of the bile acids.
- It is also believed that vitamin C is involved in the process of steroidogenesis since the adrenal cortex contains high levels of vitamin C, which are depleted upon adrenocorticotropic hormone (ACTH) stimulation of the gland.

Vitamin C is found in fresh fruits and vegetables including citrus fruits. Vitamin C is necessary for the health of the supporting tissues of the body such as bone, cartilage and connective tissue.

Deficiency in vitamin C leads to the disease scurvy due to the role of the vitamin in the post-translational modification of collagens. Scurvy is characterized by easily bruised skin, muscle fatigue, soft swollen gums, decreased wound healing and hemorrhaging, osteoporosis, and anemia.

Vitamin C is readily absorbed and so the primary cause of vitamin C deficiency is poor diet and/or an increased requirement. The primary physiological state leading to an increased requirement for vitamin C is severe stress (or trauma). This is due to a rapid depletion in the adrenal stores of the vitamin. The reason for the decrease in adrenal vitamin C levels is unclear but may be due either to redistribution of the vitamin to areas that need it or an overall increased utilization.

Answer in one word

1. Which of the following foods contains the most calcium?

   Answer : One half cup sardines with bones

2. One banana contains what percent of our recommended daily allowance for potassium?

   Answer : 11 percent

3. Which fat-soluble vitamin is manufactured by the body when exposed to UV light?

   Answer : Vitamin D

4. Water-soluble vitamins must be consumed daily because

   Answer : Water-soluble vitamins are not normally stored in the body in any significant amounts.

5. Which B vitamin is especially important for anyone who may become pregnant?

   Answer : Folate

6. Which mineral is important in the regulation of metabolism and can be found in seafood, cod, cod-liver oil, halibut, oysters, and kelp.

   Answer : Iodine

7. Which mineral is best known for its role as part of haemoglobin?

   Answer : Iron

8. A deficiency of this fat-soluble vitamin may result in total blindness. Found in spinach, carrots, sweet potatoes, and cantaloupe, it is responsible for maintaining good colour vision and night vision.

   Answer : Vitamin A

9. Which vitamin is well-known for it's important function in blood clotting, has now been found to play an important role in maintaining healthy bones.

Answer : Vitamin K

10. Which vitamin is essential in carbohydrate metabolism?

Answer : Vitamin B-1

11. Which vitamin is essential for cellular growth?

Answer : Vitamin B-2

12. Which vitamin prevents development of megaloblastic anemia?

Answer : Folic Acid

13. Which vitamin protects essential fatty acids in the body?

Answer : Vitamin E

14. Which vitamin is essential to night vision?

Answer : Vitamin A

15. Which vitamin helps your body use calcium and phosphorus?

Answer : Vitamin D

16. Which vitamin is important for the integrity of the nervous system?

Answer : Vitamin B-6

17. Which vitamin assures proper development of red blood cells?

Answer : Vitamin B-12

18. Which vitamin is essential to sound bones and wound healing?

Answer : Vitamin C

19. Which vitamin is essential for tissue respiration?

Answer : Vitamin B-3 (Niacin)

20. Which vitamin play a central role in fatty acid synthesis?

Answer : Biotin

21. Which vitamin is involved in the intermediate metabolism of carbohydrates, proteins and fats?

Answer : Vitamin B 5 (Pantothenic Acid)

22. Which vitamin can prevent neural tube birth defects?

Answer : Folic acid

23. Which vitamin is one of the most promising treatments for lowering cholesterol in the blood.

Answer : Vitamin B3 (Niacin)

24. Which vitamin helps blood clot?

Answer : Vitamin K

25. Which vitamins are water-soluble?

Answer : Vitamin C and B complex

26. Which vitamin can you get from taking a stroll on a sunny day?

Answer : Vitamin D

27. Which vitamin(s) may be helpful for people with Alzheimer's disease?

Answer : Vitamins C and E

Indicate whether the sentence or statement is true (T) or false (F).

1. Starches have a high-fat content and should be kept to a minimum in your diet.
Answer : F
2. Milk and milk products should not be consumed by adults.
Answer : F
3. The more protein in your diet is better.
Answer : F
4. Taking vitamin supplements will make up for missed meals.
Answer : F
5. Snacking is a bad habit.
Answer : F
6. It's okay to skip breakfast and make up for it with a large lunch.
Answer : F
7. Beans provide a good source of protein.
Answer : T
8. Poultry skin should be removed before cooking to eliminate the greatest percentage of fat.
Answer : F
9. Frozen vegetables have less nutritional value than fresh.
Answer : F
10. The average, healthy person needs vitamin supplements.
Answer : F
11. We get vitamin D only from foods.
Answer : F
12. Cooking vegetables in small amounts of water helps limit the amounts of water-soluble vitamins lost.
Answer : T
13. Folate is a very important vitamin, especially for girls and women who are pregnant or of child-bearing age.
Answer : T
14. There are three main types of vitamins: fat-soluble, carbohydrate-soluble and water-soluble.
Answer : F

“This Page is Intentionally Left Blank”

Chapter 8

Introduction to Metabolism

The reaction pathways that comprise metabolism are divided into two categories.

- **Catabolism**: degradation of molecules to provide energy.
- **Anabolism**: reactions using energy to synthesize new molecules for growth.

In catabolic reactions, complex substances are broken down to simpler compounds with a concomitant release of free energy. The released free energy during these catabolic reactions is conserved in the form of ATP or NADPH. The major nutrients such as carbohydrates, lipids and proteins are converted to common intermediate and further metabolised in a central oxidative pathway.

The opposite process occurs during biosynthesis. Simple organic molecules such as pyruvic acid, acetyl unit or intermediate compounds of citric acid cycle serve as starting molecules for varied biosynthetic products. The energy rich molecules such as ATP or NADPH derived from catabolic reactions are utilized in the biosynthetic reactions.

Metabolic Pathways

"A metabolic pathway is a series of enzyme-catalyzed reactions, initiated by a flux-generating step and ending with either the loss of products to the environment, to a stored product (a metabolic 'sink') or in a reaction that precedes another flux-generating step (that is, the beginning of the next pathway)." Where a **flux generating step** is a non-equilibrium reaction that generates the flux going through the pathway and to whose rate all other reactions of the pathway conform. Note that by this definition some pathways may be inter-organ while others may take place in single compartment. We will explore this definition/concept as we look at metabolism.

Characteristics of Pathways

- Irreversible
- First committed step (flux generating step)
- Regulated
- Localized in eukaryotes

- Catabolic and anabolic pathways are generally distinguished by coenzymes and/or compartmentalization.

The flux through a metabolic pathway is invariably controlled or *regulated,* most commonly by *Feedback Inhibition,* but also through Feed-forward activation. Regulation is one of the things that makes biochemistry "biological" and it will be a focus in our study.

The Stages of Catabolism : For convenience, catabolism can be divided into four hierarchical levels:

- **Stage I:** Hydrolysis of polymers to monomeric units (fat ⟶ fatty acids and glycerol, protein ⟶ amino acids, etc.)
- **Stage II:** breakdown of products of Stage I to pyruvate, acetyl CoA, and/or intermediates of the Kreb's Cycle
- **Stage III:** Breakdown of Acetyl CoA by the Kreb's Cycle into carbon dioxide and water with the production of reducing equivalents (NADH etc.)
- **Stage IV:** Oxidation of the reducing equivalents by oxygen with the production of ATP via the Electron Transport System.

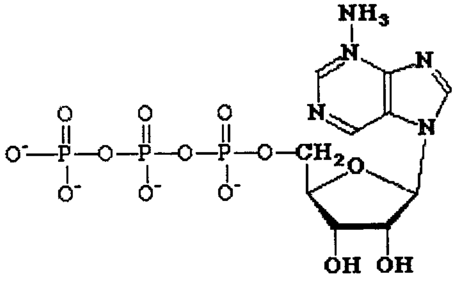
Organic Reactions in Metabolism

Organic Reaction Mechanisms: It can be categorized all common biological reactions into four groups -

- Group-transfer reactions (transfer of an electrophile [acyl {RCOX}, phosphoryl {OPO_3X^{2-}}, and glycosyl groups] between nucleophiles [alcohols, amines, thiols, etc.])
- Redox Reactions
- Eliminations {eliminate H_2O, NH_3, ROH or RNH_2}, Isomerizations, Rearrangements
- C-C bond formation or breakage (condensation and cleavage reactions).

High Energy Compounds

Look at ATP. In the figure the bolded region is the "recognition" part of the molecule, while the polyphosphate is the chemically active portion. Each of the phosphoric acid anhydride bonds is unstable. That is hydrolyzing either will release a lot of energy.



So why ATP? First, we want a compound with intermediate hydrolysis energy so it can pick up energy from some reactions and deliver to others. Second we want a **kinetically stable** molecule which is **thermodynamically unstable**. Thus acetic acid anhydride would not work: it is thermodynamically unstable to hydrolysis, but it is also kinetically unstable, with the carbonyl carbons wide open to water attack. Phosphoric acid anhydride is equally unstable, but is is sterically protected from water attack - in order to react quickly we need a catalyst - perfect.

ATP is sometimes referred to as a "High Energy" compound. High energy in this case does **not** refer to total energy in compound, rather just to energy of hydrolysis. Thus ATP is unstable to hydrolysis, or has a large negative ΔG for hydrolysis. For biochemistry *High Energy* is defined in terms of ATP: if a compound's free energy for hydrolysis is equal to or greater than ATP's then it is "High Energy," if its free energy of hydrolysis is less than ATP's then it is not a "high energy" compound. Note that ATP has two high energy anhydride bonds.

Thermodynamics in Metabolism

Thermodynamics is the study of energy and its transformations A knowledge of thermodynamics, which is the description of the relationships among the various forms of energy and how energy affects matter, enables one to determine whether a physical process is possible. The first and second law of thermodynamics are combined in the thermodynamics function, free energy (G). If the change in free energy (ΔG) of reaction is negative, that reaction can occur spontaneously. If ΔG is positive, an input of energy is required to derived the reaction. The unit of energy is jule (J) or the calorie (cal).

- Energy can be loosely defined as the ability of a system to do work on its surroundings. Work means moving things, deforming things, breaking things, etc.
- The free energy is defined as: $\Delta G = G_{products} - G_{reactants} = \Delta H - T\,\Delta S$.

- When the free energy is negative we say the reaction is spontaneous, which simply means the reactants are favoured in the reaction equation as written.
- Note when a reaction is at equilibrium then the ΔG is zero.
- The first law of thermodynamics state that the total energy of a system and it's surroundings is a constant.

  $$\Delta E = E_B - E_A = Q - W$$

  Where, EA is the energy of a system at the start of a process and EB at the end of the process. Q is the heat absorbed by the system and W is the work done by the system.
- The second law of the thermodynamics states that a process can occur spontaneously only if some of the entropies of the system and its surroundings increases (or that the universe tends towards maximum disorder), that is

  $$(\Delta S_{system} + \Delta S_{surroundings}) > 0 \text{ for aspontaneous process)}$$
- The process in which the system release heat (i.e., have a negative Q) are known as exothermic process.
- The process in which system gains heat (i.e., have a positive Q) are known as endothermic process.

Since free energy depends on conditions, chemists tabulate free energies under Standard Conditions, ($\Delta G°$): 298 K, 1 atm., with all concentrations at 1 M.

For biological systems we define a slightly different standard free energy with $[H^+]= 10^{-7}$ M (pH=7), $\Delta G°'$.

For non-standard conditions we can find the free energy of a reaction using: $\Delta G = \Delta G°' + RT \ln Q$. For the special case of equilibrium, the free energy is zero, so $\Delta G°' = -RT \ln K'$, $\Delta G°' = -5700 \log K$ (in joules). Thus free energy is related to the equilibrium constant, K.

The free energy is zero, so $\Delta G°' = -RT \ln K'$, $\Delta G°' = -5700 \ln K$ (in joules @ 25°C). Thus free energy is related to the equilibrium constant, K. To provide a quantitative feeling for this relationship some values are tabulated below as Table 8.1 :

Table 8.1 : Relationship between Free Energy and Equilibrium Constant (K)

K	log K	$\Delta G^{\circ\prime}$ (calories)	$\Delta G^{\circ\prime}$ (joules)
10^{-3}	-3	4,089	17,100
10^{-1}	-1	1,363	5,700
1	0	0	0
10^{3}	3	-4,089	-17,100

For non-equilibrium situations we can find the energy available for work using $\Delta G = \Delta G^{\circ\prime} + RT \ln Q$, where Q is the mass action expression, Q = ([C][D])/([A][B]) for the reaction $A + B \rightleftharpoons C + D$.

One advantage of using free energy is that it is easier to evaluate the overall equilibrium/energy for a series of sequential reactions (its additive instead of multiplicative): ΔG_{tot} = Sum[ΔG]. Often use to predict feasibility of pathways, possible energy yields, and to determine when individual reactions are **not** at equilibrium (important for determining potential control steps etc.).

Note that the overall free energy determines spontaneity of the reaction - the pathway doesn't matter! As noted above thermodynamics is pathway independent. Thus can drive unfavourable reactions by linking with favourable reactions. This can be done:

1. Sequentially: $A \xrightarrow{\Delta G=+} B \xrightarrow{\Delta G=--} C$, where the reaction of B to C pulls A to B; or
2. In parallel: $\begin{matrix} A \xrightarrow{\Delta G=+} B \\ C \xrightarrow[\Delta G=--]{} D \end{matrix}$, where the two reactions are linked.

Example: glucose + phosphate to G-6-P (ΔG= +3300 cal) and ATP + water to ADP + P_i (ΔG= -7600 cal); mix together, no G-6-P (ΔG= -4300 cal). But link with enzyme, Glu + ATP $\rightleftharpoons$ G-6-P + ADP (ΔG= -4300 cal). All of metabolism depends on such *coupled* reactions. In essence catabolic reactions drive anabolic reactions etc., via direct, and more commonly, indirect, multi-step, coupling.

Metabolism would be extremely complex if coupled processes directly, however. Instead use an intermediate energy carrier: ATP. Thus, catabolic processes make ATP which can then be used for anabolic processes, locomotion, pumping ions across cell membranes (major contribution to basal metabolic rate or BMR), etc. Note that ATP is **not** used to store energy however. (Often compared to electricity's role in our culture).

Overview of Meatbolism

Metabolism is the sum total of all chemical reactions involved in maintaining the living state of the cells, and thus the organism. In general metabolism may be divided into two categories: catabolism or the breakdown of molecules to obtain energy; and anabolism or the synthesis of all compounds needed by the cells (examples are DNA, RNA, and protein synthesis).

Bioenergetics is a term which describes the biochemical or metabolic pathways by which the cell ultimately obtains energy.

Nutrition is a science that deals with the relation of food substance to living things.

Essential foods supply energy (calories) and supply the necessary chemicals which the body itself cannot synthesize. Food provides a variety of substances that are essential for the building, upkeep, and repair of body tissues, and for the efficient functioning of the body.

A complete diet must supply the elements; carbon, hydrogen, oxygen, nitrogen, phosphorus, sulfur, and at least 18 other inorganic elements. The major elements are supplied in carbohydrates, lipids, and protein. In addition, at least 17 vitamins and water are necessary. If an essential nutrient is omitted from the diet, certain deficiency symptoms appear.

Carbohydrates:

Foods supply carbohydrates in three forms: starch, sugar, and cellulose (fiber). Starch and sugar are major and essential sources of energy for humans. A lack of carbohydrates in the diet would probably result in an insufficient number of calories in the diet. Cellulose furnishes bulk in the diet.

Since the tissues of the body need glucose at all times, the diet must contain substances such as carbohydrates or substances which will yield glucose by digestion or metabolism. For the majority of the people in the world, more than half of the diet consists of carbohydrates from rice, wheat, bread, potatoes, macaroni.

Proteins:

All life requires protein since it is the chief tissue builder and part of every cell in the body. Among other functions, proteins help to: make hemoglobin in the blood that carries oxygen to the cells; form anti-bodies that fight infection; supply nitrogen for DNA and RNA genetic material; and supply energy.

Proteins are necessary for nutrition because they contain amino acids. Among the 20 or more amino acids, the human body is unable to synthesize 8, therefore, these amino acids are called essential amino acids. A food containing protein may be of poor biological value if it is deficient in one or more of the 8 essential amino acids: lysine, tryptophan, methionine, leucine, isoleucine, phenylalanine, valine, and threonine. Proteins of animal origin have the highest biological value because they contain a greater amount of the essential amino acids. Foods with the best quality protein are listed in diminishing quality order: whole eggs, milk, soybeans, meats, vegetables, and grains.

Fats and Lipids:

Fats are concentrated sources of energy because they give twice as much energy as either carbohydrates or protein on a weight basis. The functions of fats are to: make up part of the structure of cells, form a protective cushion and heat insulation around vital organs, carry fat soluble vitamins, and provide a reserve storage for energy.

Three unsaturated fatty acids which are essential include: linoleic, linolinic, and arachidonic acids and have 2, 3, and 4 double bonds respectively. Saturated fats, along with cholesterol, have been implicated in arteriosclerosis, "hardening of the arteries". For this reason, the diet should be decreased in saturated fats (animal) and increased in unsaturated fat (vegetable).

As already mentioned, metabolism refers to the chemical reactions carried out inside of the cell. The major metabolic reactions which we will study are those involving catabolism which is the breakdown of larger molecules to extract energy. The overall reaction for the combustion of glucose is written:

$$C_6H_{12}O_6 + 6\ O_2 \text{ -----> } 6\ CO_2 + 6\ H_2O + \text{energy}$$

Although the above equation represents the overall metabolic reaction for carbohydrates, there are actually over thirty individual reactions. Each reaction is controlled by a different enzyme. The failure of an enzyme to function may have serious and possibly fatal consequences. Slightly less than half of the 686 kcal/mole of the energy produced by combustion is available for storage and use by the cell with the remaining amount dissipated as heat.

Metabolism will be studied in various parts. Interrelationships will be pointed out as they are encountereD. Just as there are three basic biomolecules - carbohydrates, lipids, and proteins, the metabolism of each of these will be studied individually.

Minerals:

The minerals in foods do not contribute directly to energy needs but are important as body regulators and as essential constituents in many vital substances within the body. A MINERAL is rather loosely defined as any element not normally a part of the structures of carbohydrates, proteins, and fats. More than 50 elements are found in the human body.

About 25 elements have been found to be essential, since a deficiency produces specific deficiency symptoms. All of the minerals required by the human body are probably not known at this time. Although minerals may not be part of the structures of carbohydrates, proteins, and fats, they are mixed in the foods in trace amounts during the growing process by uptake from the soil.

Major Minerals Include: calcium, phosphorus, iron, sodium, potassium, and chloride ions.

Other Essential Minerals Include: copper, cobalt, manganese, zinc, magnesium, fluorine, and iodine.

Vitamins:

Vitamins are essential organic compounds that the human body cannot synthesize by itself and must therefore, be present in the diet. The term vitamin (vital amines) was coined by Casmir Funk from the Latin vita meaning "life" (essential for life) and amine because he thought that all of these compounds contained an amine functional group.

Vitamins particularly important in metabolism include:

Vitamin A: The yellow and green pigments found in vegetables are called carotenes which are pro vitamins and are converted into Vitamin A.

Vitamin B_2 is better known as riboflavin and is widely distributed in many foods. Riboflavin is used to form a coenzyme FAD important in the utilization of oxygen in the cells.

Niacin, also known as nicotinic acid, is also in the B complex of vitamins. Nicotinic acid was first obtained from the alkaloid nicotine in tobacco and was later found in many plant and animal tissues as niacin.

Nicotinamide is a part of the important coenzyme, **Nicotinamide Adenine Dinucleotide (NAD).** This NAD^+ coenzyme is important during biological oxidations and is discussed in detail in a later page.

Pantothenic Acid is art of the structure of coenzyme A.

Flow of Energy in the Biosphere:

All of the chemical processes of the cell are called **metabolism.** The breakdown or degradation of complex organic molecules to yield simple molecules and energy is called **catabolism. Anabolism** is the total biosynthetic processes where large complex molecules are made from small simple molecules. Anabolic processes require energy because order is being created and thus work must be done. Overall, both processes of metabolism must occur concurrently because catabolism provides the energy necessary for anabolism.

Plants utilize energy from the sun in the photosynthetic process to synthesize larger molecules from smaller ones. On the other hand, animals and humans use the plants for fooD. The larger molecules are catabolized to provide energy.

The definition for an **exothermic reaction** is one where energy is given off. An **endothermic reaction** is where energy is required.

Uses of Energy in the Cells

The body and cells need a constant supply of energy for a variety of reasons. Energy is needed to carry out mechanical work which involves the change in location or orientation of a body part or the cell itself. A major example is the energy required for the contraction of muscles.

Molecular transport also requires energy. The movement of molecules from an area of low concentration to an area of higher concentration requires energy since this is opposite to the normal movement of molecules. This process is also called active transport. Examples include the movement of nutrient raw materials into a cell and the movement of waste materials out of the cell.

Electrical work is also included under molecular transport since the establishment of a differential concentration of ions across a membrane is used to build up an electrical charge. The result of electrical work is the excitation and conduction of impulses in nerve and muscle cells.

Finally, energy is needed for the synthesis of new complex biochemical molecules. Biosynthesis involves the formation of many new molecules from simpler molecules. New cellular material is produced not only during active periods of growth but also in existing structures to repair and replace damaged molecules.

QUIZ

Give Answer in brief

Q-1 What is energy and whjy is it required by cells? (ans)

A-1 Energy is the ability jto do work. Energy is required to drive various biosynthetic chemical reactions and do mechanical work.

Q-2 The energy found within tjhe chemical bonds of nutrients and ATP is an example of :

A-2 Potential energy because potential energy is stored energy such as the energy stored in the chemical bonds of carbohydrates, lipids, proteins, and ATP.

Q-3 During photosynthesis, cells of plants and algae create energy from sunlight- True or False ?

A-3 False, the first law of thermodynamics states that energy can be neither created nor destroyed. It can, however, be converted from one form to another.

Q-4 During photosynthesis, the energy in photons from the sun are converted into the energy in the chemical bonds of glucose and other organic molecules. During cellular respiration, the energy in the chemical bonds of glucose and other organic molecules can then be converted into energy in the chemical bonds of ATP, the form of energy most commonly used to do cellular work. The ability to do this is based on what ?

A-4 The first law of thermodynamics which states that energy can be neither created nor destroyed. It can, however, be converted from one form to another.

Q-5 After exercising, you body feels hot. Explain this in terms of the laws of thermodynamics.

A-5 The second law of thermodynamics states that when energy is converted from one form to another, some energy is always lost as heat. In other words, no energy conversion is ever 100% efficient. Some usable energy, the energy available to do work, is always dispersed as heat into the surrounding environment. As the energy in the bonds of ATP is used for muscle movement, much is lost as heat.

Q-6 The synthesis of DNA and proteins is an example of:

A-6 An anabolic reaction - Biochemical reactions that require energy during the synthesis of chemical compounds are called anabolic reactions

Q-7 In glycolysis, one molecule of glucose is converted into two molecules of pyruvate, 2 NADH + 2H+, and two net ATP. This is an example of:

A-7 A catabolic pathway - Biochemical reactions that harvest energy during the breakdown of chemical compounds are called catabolic reactions.

Q-8 The energy-requiring synthesis of proteins from amino acids is an example of:

A-8 An endergonic reaction - Biochemical reactions that require an input of energy are said to be endergonic reactions.

Q-9 Organisms that carry out use light as an energy source and carbon dioxide as their main carbon source are called:

A-9 Photoautotrophs - Photoautotrophs use light as an energy source and carbon dioxide as their main carbon source. They include photosynthetic bacteria (green sulfur bacteria, purple sulfur bacteria, and cyanobacteria), algae, and green plants.

Q-10 Organisms that use organic compounds as both an energy source and a carbon source are called:

A-10 Chemooganoheterotrophs (ans) - Chemooganoheterotrophs use organic compounds as both an energy source and a carbon source. Most bacteria, and all protozoans, fungi, and animals are chemoorganoheterotrophs

Q-11 Describe how cells trap energy released from exergonic catabolic chemical reactions and store it as ATP.

A-11 ATP- To trap energy released from exergonic catabolic chemical reactions, the cell uses some of that released energy to attach an inorganic phosphate group on to adenosine diphosphate (ADP) to make adenosine triphosphate (ATP). Thus, energy is trapped and stored in what are known as high-energy phosphate bonds.

Q-12 Describe how cells obtain energy to do cellular work during endergonic anabolic chemical reactions.

A-12 ATP: To obtain energy to do cellular work during endergonic anabolic chemical reactions, the organism enzymatically removes the thirdphosphate from ATP thus releasing the stored energy and forming ADP and inorganic phosphate.

Q-13 The hydrolysis of ATP is:

A-13 An exergonic reaction where energy is released.

Identify the letter of the choice that best completes the statement or Answers the question.

1. Catalysts:
    A. slow down chemical reactions
    B. are used up in reactions
    C. provide an alternative reaction pathway
    D. increase the activation energy

Answer : C

2. The conversion of glucose to carbon dioxide and water is an example of:
    A. a catabolic reaction.
    B. a condensation reaction
    C. an esterification reaction
    D. an anabolic reaction

Answer : A

3. Which of the following is *not* a feature of collision theory?
    A. the rate of chemical reactions increases with increasing temperatures
    B. the more molecules present, the faster the reaction
    C. the reaction is faster in dilute solute solutions than in concentrated
    D. at high temperatures molecules have more energy than at low temperatures

Answer : C

4. Examples of anabolic reactions include:
    A. hydrolysis reactions
    B. the breakdown of carbohydrates
    C. the breakdown of lipids
    D. the build up of proteins

Answer : D

5. In an exergonic reaction:
    A. bonds being formed are the same strength as bonds being broken
    B. bonds being formed are stronger than bonds being broken
    C. energy is absorbed from the surroundings
    D. energy is released to the surroundings

Answer : D

6. In an endergonic reaction:
    A. bonds being formed are the same strength as bonds being broken
    B. bonds being formed are stronger than bonds being broken
    C. energy is absorbed from the surroundings
    D. energy is released to the surroundings

Answer : C

7. In the water cycle, photosynthesis:
    A. converts liquid water into solid water

B. converts glucose into water

C. fixes hydrogen from water into biomass

D. converts gaseous water to liquid water

Answer : C

8. The main reason why green plants cannot use nitrogen directly from the air is:

A. the triple bonds holding the nitrogen atoms together in the molecule require too much energy to break them

B. nitrogen molecules are insoluble in water

C. nitrogen gas dissolves in water to produce a strongly acidic solution which would damage the plant cells

D. the nitrogen molecule is too unstable

Answer : D

9. A system's productivity is measured in:

A. $kJ\ m^{-1}\ yr^{-1}$

B. $kJ\ m^{-2}\ yr^{-1}$

C. $kJ\ m\ s^{-1}$

D. $kJ\ m^{-2}\ s^{-1}$

Answer : B

10. Gross primary production is highest in:

A. tundra

B. a rainforest

C. prairie

D. a desert

Answer : B

11. In the carbon cycle, photosynthesis:

A. releases carbon dioxide into the atmosphere

B. releases carbon dioxide from the oceans

C. fixes carbon in biomass

D. fixes carbon in carbonates

Answer : C

12. When light energy is absorbed by chlorophyll during photosynthesis:

A. chlorophyll combines with carbon dioxide to produce glucose

B. chlorophyll decomposes to form glucose and water

C. chlorophyll is converted to ATP

D. a high energy electron is released from the chlorophyll molecule

Answer : D

13. In green plants, energy is stored mainly in:

A. glucose

B. ATP

C. starch

D. cellulose

Answer : C

14. The rate at which energy from sunlight is made available to consumers by green plants is known as a system's:
    A. net primary production
    B. productivity
    C. growth rate
    D. ross primary production

Answer : A

15. Chemical reactions that release energy in the cell are _________ reactions.
    A. endergonic B. exergonic C. energetic D. relaxed

Answer : B

16. What is a type of reaction that takes place in the absence of oxygen?
    A. anaerobic reaction B. energetic reaction
    C. aerobic reaction D. transport reaction

Answer : A

17. What molecule results when ATP undergoes an exergonic reaction with water?
    A. adenine triphosphate B. adenine phosphate
    C. adenosine diphosphate D. adenosine triphosphate

Answer : C

18. What is the molecule most readily available for energy in animals?
    A. lipid B. starch C. glucose D. fructose

Answer : C

19. Overall, the process of photosynthesis is a(n) _________ reaction.
    A. endergonic B. exergonic
    C. exothermic D. synthetic

Answer : A

20. The reactions of photosynthesis can be described by the following word equation: six molecules of carbon dioxide and six molecules of water react in the presence of sunlight with enzymes to yield one molecule of simple sugar and six molecules of ___________ .
    A. carbon B. oxygen C. hydrogen D. ozone

Answer : B

21. Chemical reactions that require free energy are _________ reactions.
    A. exergonic B. endergonic C. relaxed D. energetic

Answer : B

22. What is the relationship between photosynthesis and cellular respiration?
    A. Both require sunlight for energy to begin the process.
    B. They are not related chemically.

C. They are interdependent processes that are the opposite of each other.
D. They are similar processes that produce energy in organisms.

Answer : C

23. The Gibb's free energy, DG, is negative for
A. exergonic processes.
B. endergonic processes.
C. temperature-independent processes.
D. None

Answer : A

24. Since DG° = -RTlnK
A. a 10-fold increase in K decreases DG° by about 10-folD.
B. a 10-fold decrease in K decreases DG° by about 2.3*RT.
C. a 10-fold increase in K decreases DG° by about 2.3*RT.
D. a 10-fold decrease in K increases DG° by about 10-folD.

Answer : C

25. If the enthalpy change for a reaction is zero, DG° is equal to:
A. TDS°.
B. -TDS°.
C. DH°.
D. -DH°.

Answer : B

26. The carboxyl group of alanine has a lower pK_a than that of acetic acid because
A. acetic acid has only two carbons.
B. DpK_a = -DG°
C. acetic acid does not have a chiral carbon.
D. the positively charged amino group facilitates proton dissociation.

Answer : D

27. The "internal energy" of a system:
A. depends only on the present state of the system
B. depends on the pathway by which the system came to its present condition
C. does not change if energy flows in or out of the system
D. is not a state function

Answer: A

28. The second law of thermodynamics states that:
A. the entropy of a system plus surroundings is unchanged by irreversible processes
B. systems tend to proceed from disordered to more ordered states

C. naturally occurring processes proceed to a state of minimum potential energy
D. the entropy of a system plus surroundings is always changed by reversible processes

Answer: C

29. For a reaction to proceed spontaneously:
A. DH must be negative. B. DS must be negative
C. DG must be negative. D. DT must be positive.

Answer: C

30. The hydrolysis of ATP above pH 7 is entropically favored because
A. The electronic strain between the negative charges is reduced.
B. The released phosphate group can exist in multiple resonance forms.
C. There is an increase in the number of molecules in solution
D. There is a large change in the enthalpy.

Answer: C

31. The free energy of hydrolysis of ATP is:
A. unaffected by the concentration of metal ions
B. increased when metal ions such as magnesium are present
C. decreased when metal ions such as magnesium are present
D. unaffected by pH

Answer: B

32. Special energized biomolecules such as ATP and NADP represent chemically useful forms of stored energy because:
A. They are extremely stable.
B. They are extremely unstable
C. When they react with other molecules, they extract energy from these molecules
D. When they react with other molecules, the energy released can be used to drive unfavorable processes.

Answer: D

Complete each sentence or statement.

1. ________ describes the degradative metabolic reactions in which nutrients and cell constituents are broken down for energy and raw materials.

Answer : Catabolism

2. A compound containing an ester linkage to a sulfur rather than an oxygen atom is a/an ________.

Answer : thioester

3. An organism that obtains its building materials and free energy from organic compounds produced by other organisms is a/an ________.

Answer : heterotroph

4. A/An ________ is an amino acid, fatty acid, or other compound that an animal cannot synthesize and must therefore obtain in its diet.

Answer : essential compound or essential nutrient

5. A/An ________ is a globular particle, containing lipids and proteins, that transports lipids between tissues via the bloodstream.

Answer : lipoprotein

6. The total of all degradative and biosynthetic cellular reactions is ________.

Answer : metabolism

7. ________ is the force that drives reactants toward their equilibrium values when the system is in its biochemical standard state.

Answer : Standard free energy change

8. An organism that obtains its building supplies and free energy from inorganic compounds is a/an _______.

Answer : chemotroph or autotroph

9. The cleavage of a chemical bond by the substitution of a phosphate group rather than water is called _______.

Answer : phosphorolysis

10. A series of enzyme-catalyzed reactions by which one substance is transformed into another is a/an ________.

Answer : metabolic pathway

11. ________ is the ratio of the product of the concentrations of reaction products to that of the reactants.

Answer : Mass action ratio

12. A precursor in the synthesis of another molecule or a product of the degradation of a molecule is a/an ________.

Answer : intermediate

13. Mobilization is the process in which polysaccharides, triacylglycerols, and proteins are degraded to make ________ available.

Answer : metabolic fuels

14. ________ is a multiprotein complex with a hollow cylindrical core in which cellular proteins are degraded to peptides in an ATP-dependent process.

Answer : Proteasome

15. The process by which the free energy obtained from the oxidation of metabolic fuels is used to generate ATP is ________.

Answer : oxidative phosphorylation

16. A/An ________ is an organism that obtains its building supplies from inorganic compounds and its free energy from sunlight.

Answer : photoautotroph

17. ________ describes the reactions by which biomolecules are synthesized from simpler components.

Answer : Anabolism

18. A membrane-bounded organelle that contains a battery of hydrolytic enzymes that digest ingested material and recycle cell components is a/an ________.

Answer : lysosome

19. The ________ is the ratio, at equilibrium, of the product of the concentrations of reaction products to that of the reactants.

Answer : equilibrium constant

20. A reaction in which a substance gains electrons is a/an ________ reaction.

Answer : reduction

21. The interaction of different signal-transduction pathways through activation of the same signaling components are called ________.

Answer : cross-talk

22. ________ is a disease caused by a deficiency of insulin or the inability to respond to insulin.

Answer : Diabetes mellitus

23. Substances that are secreted by one tissue into the bloodstream and that induce a physiological response in other tissues are ________.

Answer : hormones

24. The ________ is a metabolic pathway in which lactate produced by glycolysis in the muscles is transported via the bloodstream to the liver, where it is used for gluconeogenesis.

Answer : Cori cycle

25. ________ is the degradation of a triacylglycerol so as to release fatty acids.

Answer : Lipolysis

26. A binding protein that is specific for its ligand and elicits a discrete biochemical effect when its ligand is bound is a/an ________.

Answer : receptor

27. An intracellular ion or molecule that acts as a signal for an extracellular event such as ligand binding to a cell surface receptor is a/an ________.

Answer : second messenger

28. ________ is the generation of heat by muscular contraction or by metabolic reactions.

Answer : Thermogenesis

29. A guanine nucleotide-binding and -hydrolyzing protein that is inactive when it binds GDP and active when it binds GTP is a/an ________.

Answer : G protein

30. ________ is a metabolic pathway in which pyruvate produced by glycolysis in the muscles is converted to alanine and transported to the liver, where it is converted back to pyruvate for gluconeogenesis.

Answer : Glucose-alanine cycle

31. An enzyme that hydrolyzes phosphoryl groups attached to proteins is ________.

Answer : phosphatase

32. Levels of glucose in the blood are elevated in the condition known as ________.

Answer : hyperglycemia

33. The inability of cells to respond to insulin is called ________.

Answer : insulin resistance

34. The kinase-catalyzed phosphorylation of itself or an identical molecule is called ________.

Answer : autophosphorylation

35. The process by which an extracellular signal is transmitted to the cell interior by binding to a cell surface receptor such that binding triggers a series of intracellular events is called ________.

Answer : signal transduction

36. A/An ________ is a small molecule that binds to a larger molecule.

Answer : ligand

37. A signal transduction pathway in which hormone binding to a cell surface receptor induces phospholipase C to catalyze the hydrolysis of phosphatidylinositol bisphosphate to yield the second messengers inositol trisphosphate and diacylglycerol is the ________.

Answer : phosphoinositide signaling system

38. An enzyme that catalyzes the transfer of a phosphoryl group from ATP to the OH group of a protein Ser, Thr, or Tyr residue is ________.

Answer : protein kinase

Chapter 9

Carbohydrate Metabolism

Cells mainly in the form of glucose utilize carbohydrate. The three principal monosaccharides resulting from the digestive processes are glucose, fructose and galactose. Both fructose and galactose are readily converted to glucose by the liver. Pentose sugars such as xylose, arabinose and ribose may be present in the diet, but their fate after absorption is obscure. Since glucose is the compound formed from starch and glycogen, the carbohydrate metabolism commences with this monosaccharide.

The major metabolic processes in carbohydrates are: (Fig. 9.1)

- **Glycolysis**: splits glucose to pyruvate, which can be converted to lactate under anaerobi condition.
- **Gluconeogenesis**: converts pyruvate to glucose.
- **Glycogenesis**: synthesis of glycogen, carbohydrate fuel storage form.
- **Glycogenolysis**: breakdown of glycogen.
- **Pentose Phosphate Pathway (PPP) or HMP pathway**: produces NADPH for cell biosynthesis.
- **Kreb's TCA cycle or Citric Acid Cycle**: converts Acetyl CoA to CO_2 and energy

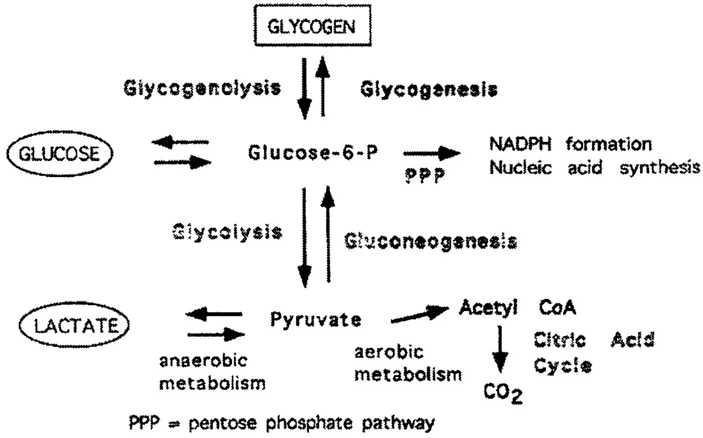

GLYCOGEN
Glycogenolysis
Glycogenesis
GLUCOSE
Glucose-6-P
PPP
NADPH formation
Nucleic acid synthesis
Glycolysis
Gluconeogenesis
LACTATE
Pyruvate
Acetyl CoA
Citric Acid Cycle
anaerobic metabolism
aerobic metabolism
CO2
PPP = pentose phosphate pathway

Fig. 9.1 : Overviews of Glucose Metabolism

Glycolysis is going to be our first pathway, and it is arguably the most important and universal of the metabolic pathways. But before we begin glycolysis let's take a brief look at how glucose (and carbohydrate generally) gets to the tissue from food intake.

Digestion

- Initial, limited, breakdown of starch in mouth by salivary amylase.
- Low pH of stomach inactivates salivary amylase, denatures many proteins etc.
- In duodenum digestive enzymes are added from the pancreas (pancreatic amylase, lipases, proteases), pH is neutralized, and detergents (bile acids) are added from gall bladder.
- Starches, Proteins, Lipids are all hydrolyzed in duodenum and small intestine where monomers are absorbed across intestinal epithelium. Amino acids and sugars are released into the portal vein to travel to the liver and then to the rest of the body.
- First pass through the liver removes sugars and most of the amino acids for processing, storage with the release of glucose and fatty acids over time for use in the peripheral tissues.

Glycolysis

Glycolysis, also called as Embden-Meyerhof-Parnas pathway (EMP pathway). Glycolysis is of central importance to the metabolism of eukaryotic cells. It links the metabolism of sugars to that of organic acids in the Krebs cycle, and in anaerobic organisms, provides the principle route of energy (ATP) generation. The reactions are rather complex, but can be seen as four basic processes (Fig. 9.2):

1. Isomerisation (catalysed by an isomerase): the intramolecular rearrangement of a molecule. This may be the transfer of a carboxyl group (C=O) from the end of a molecule (such as an aldose) to the middle (such as a ketose), or it may be the transfer of a phosphate group (the enzymes catalysing this latter sort of isomerisation are generally termed mutases).
2. Phosphorylation (catalysed by kinases): the movement of a phosphate group from one molecule (such as a phosphorylated sugar) to another (such as ATP).
3. Dehydration (catalysed by a dehydratase or hydrolase acting in reverse): the removal of water from a molecule.
4. Aldol cleavage (catalysed by an aldolase): the splitting of the carbon-carbon bond in a -(C=O)-CH(OH)-CH(OH)- molecule to generate a free aldehyde.

Many of these reactions are freely reversible, so the glycolytic pathway can (for the most part) be run in either direction for the most part.

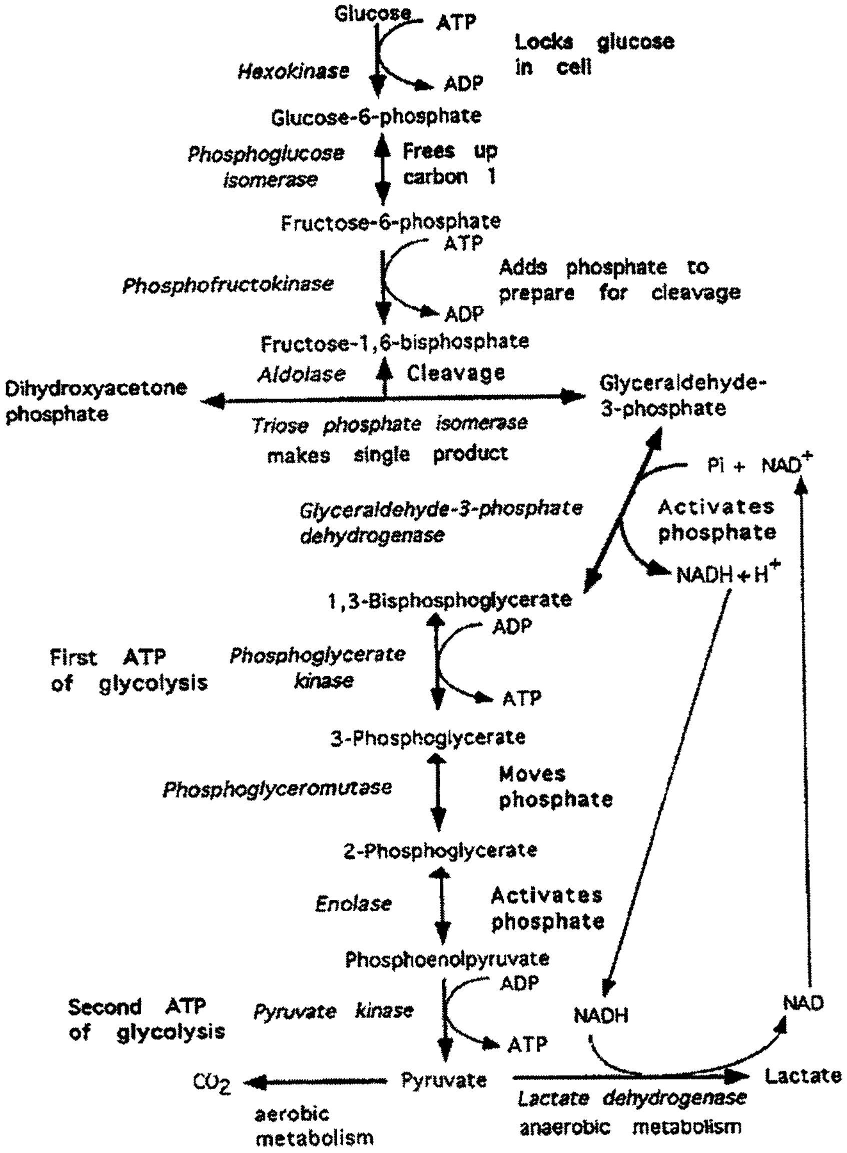

Glucose
ATP
Hexokinase
ADP
Locks glucose in cell
Glucose-6-phosphate
Phosphoglucose isomerase
Frees up carbon 1
Fructose-6-phosphate
ATP
Phosphofructokinase
ADP
Adds phosphate to prepare for cleavage
Fructose-1,6-bisphosphate
Aldolase
Cleavage
Dihydroxyacetone phosphate
Glyceraldehyde-3-phosphate
Triose phosphate isomerase makes single product
Pi + NAD+
Glyceraldehyde-3-phosphate dehydrogenase
Activates phosphate
NADH + H+
1,3-Bisphosphoglycerate
ADP
First ATP of glycolysis
Phosphoglycerate kinase
ATP
3-Phosphoglycerate
Phosphoglyceromutase
Moves phosphate
2-Phosphoglycerate
Enolase
Activates phosphate
Phosphoenolpyruvate
ADP
Second ATP of glycolysis
Pyruvate kinase
ATP
NADH
NAD
CO2
Pyruvate
Lactate
aerobic metabolism
Lactate dehydrogenase
anaerobic metabolism

Fig. 9.2. : Glycolysis Pathway

Table 9.1 : Free energies, apparent equilibrium constants, mass action ratios, and maximum enzyme activities (in micromol S transformed/min/g fresh tissue) for glycolytic enzymes [Taken from Newsholme and Start, Regulation in Metabolism, Wiley (1973)].

Glycolytic Enzymes	$\Delta G^{\circ\prime}$ (kJ)	K'	Brain		Skeletal Muscle		RBC	
			Q	Max Act	Q	Max Act	Q	Max Act
Hexokinase	-21.94	5000	0.04	17	-	1.5	0.00076	0.3
Hex.Isomerase	2.36	0.4	0.22	80	-	176	0.41	5.6
PFK	-17.80	1000	0.13	24	-	56	0.044	1.8
Aldolase	23.73	0.0001	0.000002	15	-	78	0.000014	0.7
Triose Isom.	8.29	0.04	-	415	-	2650	0.35	97
GAP DH	-	-	-	105	-	440	-	17.1
PGA K	-	-	-	610	-	169	-	25.6
DH+K	-17.22	800	53	-	-	-	124	-
Mutase	4.89	0.15	0.1	122	-	100	0.15	8.6
Enolase	-3.23	3.5	3.6	47	-	158	1.7	1.6
Pyr K	-23.73	10000	5.4	164	-	387	51	4.6
Lactate DH	-	-	-	100	-	366	-	20.4

K' gives equilibrium values under standard conditions, while Q gives measured values for real tissues (Table 9.1). Attention should be paid for differences between these two values (small variations are expected since tissues are not at standard conditions). Here large differences indicate reactions which are **not at equilibrium**: these reactions must be controlled in some way by the organism! Thus, it can be seen that large differences for HK, PFK, and PK in brain, and HK and PFK in RBC's. Muscle is like brain. The ΔG values are plotted below as well for clarity. Finally the max activity column shows what kind of flux is possible through these enzymes - what does this indicate about these tissues and glycolysis?.

As per the glycolysis pathway, the energy drops in the free energy plot were as shown below: (Fig. 9.3)

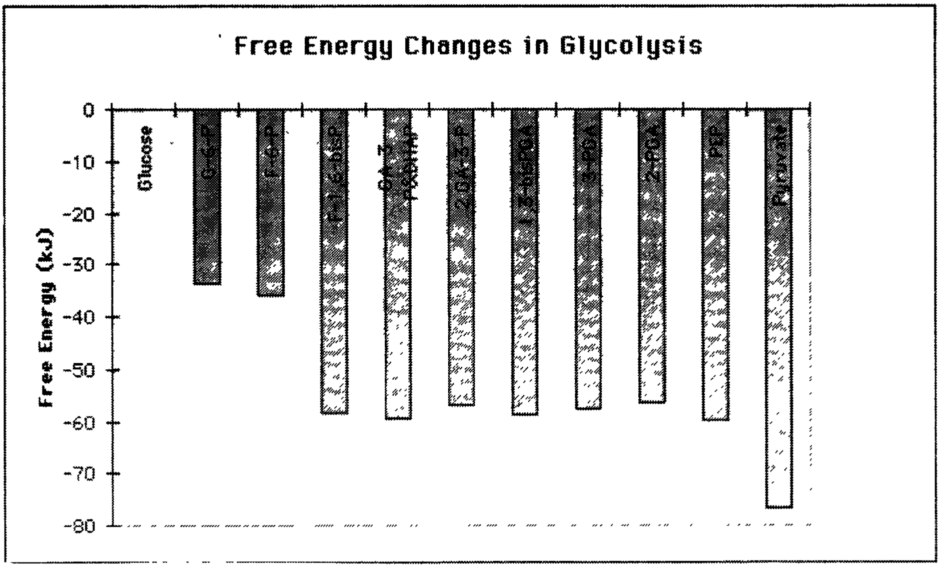
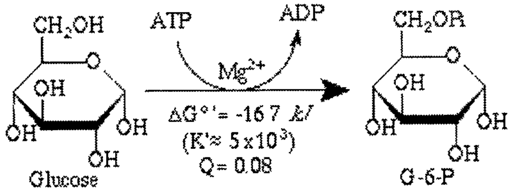


Fig. 9.3 : Free Energy Changes during Glycolysis

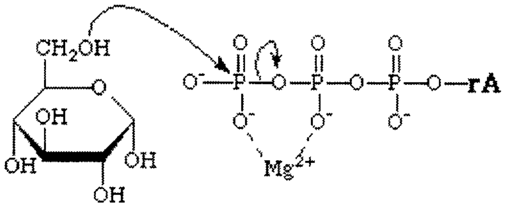
Free Energy changes in rabbit skeletal muscle [Data from Mathews and van Holde, *Biochemistry*, Benjamin/Cummings (1990)].

Individual Reactions of Glycolysis.

1) Hexokinase (HK): Glucose to G-6-P.

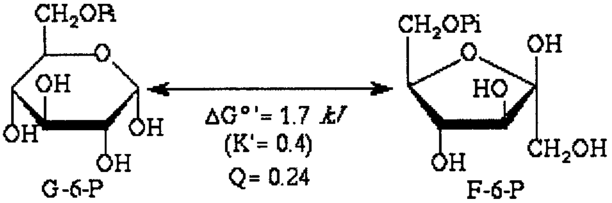

CH₂OH
O
OH
OH
OH
OH
Glucose
ATP
ADP
Mg²⁺
ΔG°'= -16.7 kJ
(K'≈ 5x10³)
Q= 0.08
CH₂OR
O
OH
OH
OH
OH
G-6-P

Nucleophilic attack by a primary alcohol on the gamma phosphate of ATP (alcoholysis of an acid anhydride). This is a very favourable reaction.

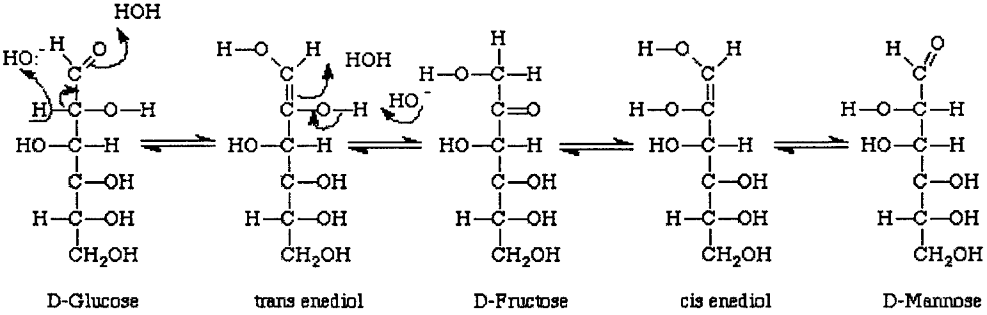

CH₂OH
O
OH
OH
OH
OH
O
O
O
O⁻
P
O
P
O
P
O
rA
O⁻
O⁻
O⁻
Mg²⁺

- Iinvolvement of magnesium in this reaction - it is an essential cofactor. (Non-Mg ATP is a potent inhibitor: What kind?).
- G-6-P inhibits this enzyme (product feedback inhibition), whereas P_i activates.
- HK is an excellent example of induced fit,

2) G-6-P Isomerase: G-6-P to F-6-P.

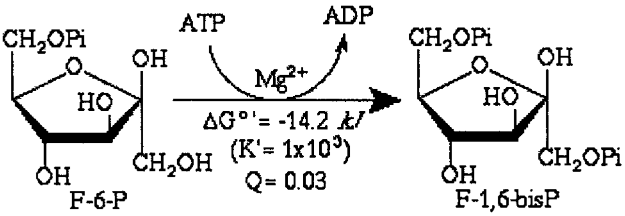

CH₂OPi
OH
OH
OH
OH
G-6-P
ΔG°'= 1.7 kJ
(K'= 0.4)
Q= 0.24
CH₂OPi
OH
HO
OH
CH₂OH
F-6-P

The mechanism here is based on the Lobry-de-Bruyn von Ekenstein mechanism.


HOH
HO⁻
HOH
HO⁻
D-Glucose
trans enediol
D-Fructose
cis enediol
D-Mannose

This would seem an ideal reaction to catalyze with a general acid/base mechanism. The enzyme has a bell shaped pH profile with pK_a's at 7 & 9 and has his and lys residues in the active site.

3) Phospho FructoKinase (PFK)-1: F-6-P to F-1,6-*bis* P.


CH₂OPi
OH
HO
OH
CH₂OH
F-6-P
ATP
ADP
Mg²⁺
ΔG°'= -14.2 kJ
(K'= 1x10³)
Q= 0.03
CH₂OPi
OH
HO
OH
CH₂OPi
F-1,6-bisP

The chemical mechanism here will be the same as for HK. However, requirement for Magnesium, as expected.

PFK is the key regulatory enzyme for Glycolysis: note it regulates the flux into the pathway and is the **first committed step** for Glycolysis.

Phosphofructokinase Regulation I

PFK is the key regulatory enzyme for Glycolysis: It regulates the flux into pathway and is the **first committed step** for Glycolysis.

ATP inhibits, giving *sigmoidal kinetics* for F-6-P vs. rate. But [ATP] is **not** important for regulation! (Probably left over from early regulatory system, but under physiological conditions [ATP] doesn't change enough to regulate PFK in most organisms. By the time [ATP] falls significantly, organism is dead).

AMP releases ATP inhibition, and is an important regulator for mammals (lots of phylogenetic variation).

Why AMP? [ATP]:[AMP] = approx. 50, while [ATP]:[ADP] = approx. 10. Thus [AMP] changes more and is much more sensitive measure of [ATP] change and thus availability (e.g. a change of about 10% in [ATP] will result in a change of about 400% in [AMP]!). Of course the problem is where does the AMP come from? Turns out there is an enzyme in most tissues catalyzing the interconversion of ATP, ADP and AMP, Adenylate Kinase:

$$2\,\mathrm{ADP} \overset{\mathrm{Mg}^{2+}}{\rightleftharpoons} \mathrm{ATP} + \mathrm{AMP}$$

An important consideration is then to determine a measure of energy in the cell. A common measure is **Energy Charge** (EC):

$$\mathrm{EC} = \frac{[\mathrm{ATP}] + 0.5\,[\mathrm{ADP}]}{[\mathrm{ATP}] + [\mathrm{ADP}] + [\mathrm{AMP}]}$$

Most cells maintain EC at a constant value with very little variation: as EC drops, catabolic, energy producing pathways, such as Glycolysis, increase in rate, while anabolic, energy consuming pathways decrease in rate. The opposite occurs as EC increases, resulting in a tight control around an optimal value, the cross-over Energy Charge, as seen in the figure: 9.4

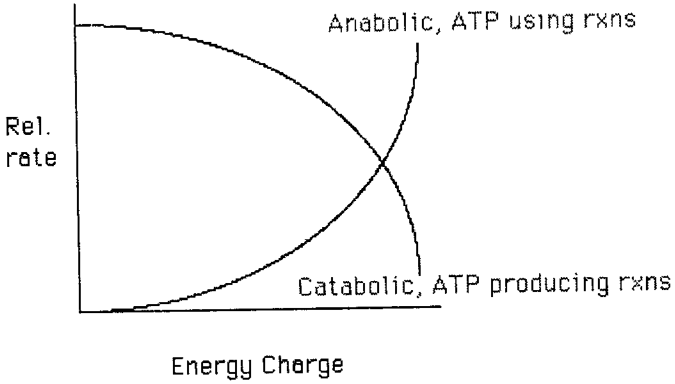


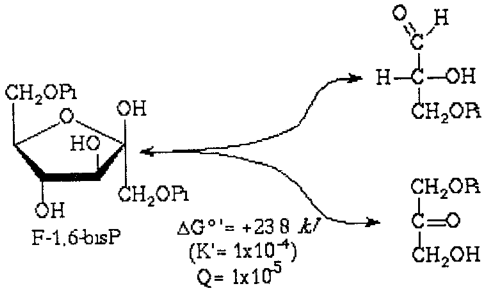
Fig. 9.4 : Energy change during Catabolic and Anabolic Activity at Cellular Level

The next reaction involves an almost symmetrical cleavage of F-1,6-*bis*P to begin **phase II**.

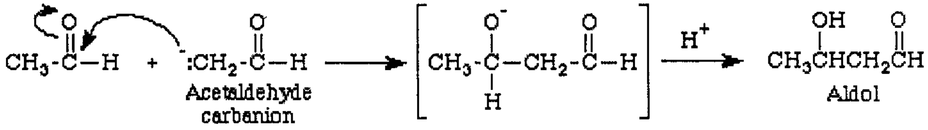
Irreversible Reactions: HK and PFK both catalyze biologically *irreversible* reactions. That is the enzymes are designed such that the concentrations of the products are far below the K_M values under physiological conditions, so the reverse reactions are not catalyzed!

The next reaction involves an almost symmetrical cleavage of F-1,6-*bis*P to begin **phase II**:

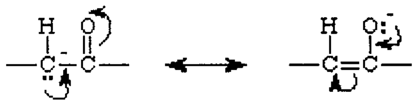
4) Aldolase: F-1,6-*bis* P to Glyceraldehyde-3-Phosphate & Dihydroxyacetone Phosphate (DHAP)


O
H
H—C—OH
CH₂OPi
CH₂OPi
O
OH
HO
CH₂OPi
OH
F-1,6-bisP
ΔG°'= +23 8 kJ
(K'= 1x10⁻⁴)
Q= 1x10⁵
CH₂OPi
C=O
CH₂OH

This reaction is an aldol cleavage.

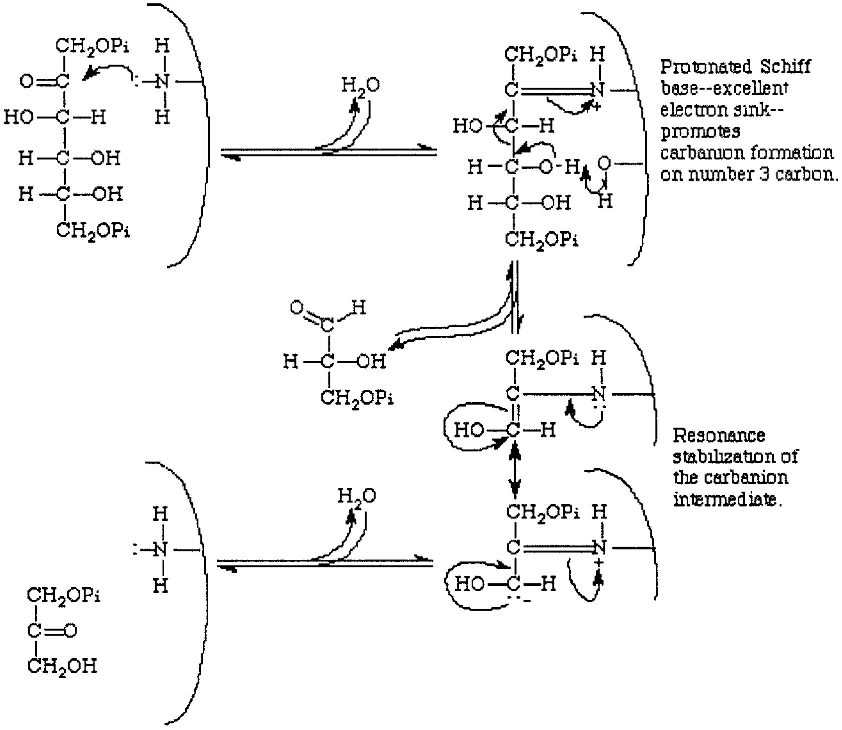


C-C bond making or breaking takes place, is only possible because of the acidity of C-2 (the C), which allows the formation of the nucleophilic carbanion. This acidity can be explained by the resonance structures which may be drawn for the alkyl-carbonyl "group":



Thus, in thinking about a catalytic mechanism for this reaction, it should be looked for ways to further stabilize the carbanion, making it an even better leaving group, and therefore, making the transition state easier to achieve.

The molecular mechanism for this enzyme, a type I aldolase, is shown below:

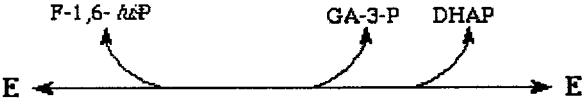


Protonated Schiff base--excellent electron sink--promotes carbanion formation on number 3 carbon.

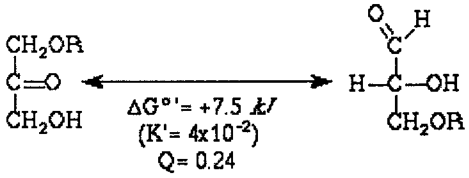
Resonance stabilization of the carbanion intermediate.

The enzyme works on the open form of the sugar, and uses a **protonated schiff base intermediate** at the heart of the mechanism.

The enzyme shows an **Ordered Sequential Uni Bi kinetic mechanism**:


F-1,6-bisP
GA-3-P
DHAP
E
E

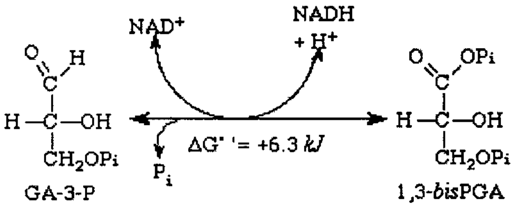
5) Triose Phosphate Isomerase: DHAP to GA-3-P


CH₂OR
C=O
CH₂OH
ΔG°'= +7.5 kJ
(K'= 4x10⁻²)
Q= 0.24
O H
C
H—C—OH
CH₂OR

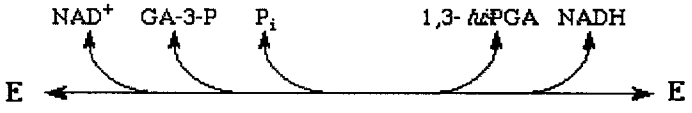
DHAP is more stable, so most of the aldolase product ends up in the DHAP pool in the cell. Need a high activity enzyme to assure the availability of this pool for proceeding through Glycolysis.

TPI turns out to have a very high turnover number (number of molecules processed per active site per time): approx. 1,000,000 mol/min/site, apparently diffusion controlled. That is this enzyme appears to operate as fast as physically possible: as soon as substrate arrives it is converted. Sometimes referred to as a "perfect catalyst."

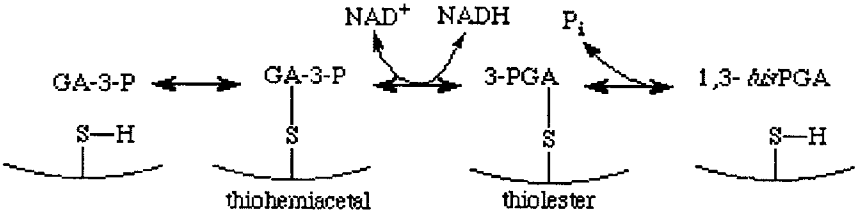
6) Glyceraldehyde 3-Phosphate Dehydrogenase: GA-3-P to 1,3-*bis* PGA


NAD⁺
NADH
+ H⁺
O H
C
H—C—OH
CH₂OPi
GA-3-P
Pi
ΔG°'= +6.3 kJ
O OPi
C
H—C—OH
CH₂OPi
1,3-bisPGA

GA-3-P DH shows an **Ordered Sequential Ter Bi kinetic mechanism**:

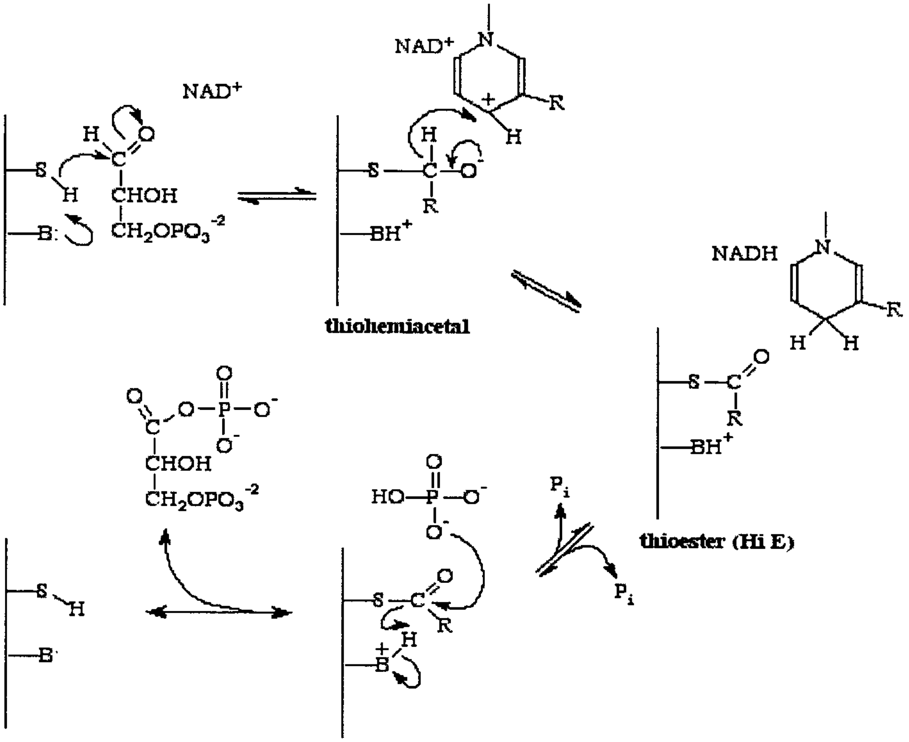

NAD⁺
GA-3-P
Pi
1,3-bisPGA
NADH
E
E

Oxidizing an aldehyde to an 'acid,' creating a mixed acid anhydride in the process:. The thiolester can be phosphorylized to give 1,3-*bis* PGA:


NAD+
NADH
Pi
GA-3-P
GA-3-P
3-PGA
1,3-bisPGA
S—H
S
S
S—H
thiohemiacetal
thiolester

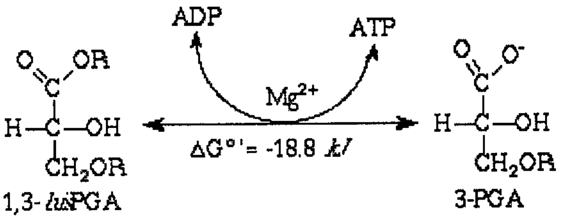
In this mechanism, the thiol group of cysteine is used both as catalyst and to preserve and transfer the free energy of the oxidation reaction. Thus, the carbon of the thiohemiacetal is less (+) than an acetal carbon and so it is easier to remove a hydride ion using NAD+, and the resulting thiol ester is a high energy compound which is readily attacked by phosphate.

Glyceraldehyde 3-Phosphate Dehydrogenase Mechanism


NAD+
NAD+
thiohemiacetal
NADH
thioester (Hi E)
Pi
Pi
CHOH
CH2OPO3-2
BH+
R
HO—P—O-

(Note: Arsenate can substitute for phosphate forming highly unstable 1-As-3-PGA, which readily hydrolyses, thus producing no ATP - one mechanism of As toxicity.).

7) Phosphoglycerate Kinase: 1,3-*bis* PGA to 3-PGA

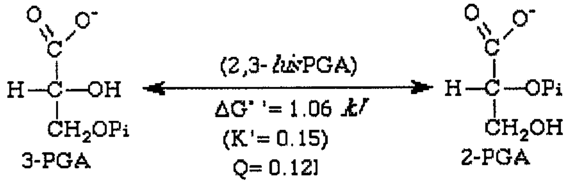


After a series of unfavourable or marginal reactions now we get a highly favorable reaction again - pulling the pathway forward.

These two reactions couple an oxidation (favourable) to a phosphorylation (unfavourable) to give a substrate level oxidative phosphorylation with the capture of the oxidative energy as ATP.

Note that the energy investment in Stage 1 has now been "paid back" and Glycolysis is now energy neutral. This brings us to the **next stage** of Glycolysis and our ATP energy "profit."

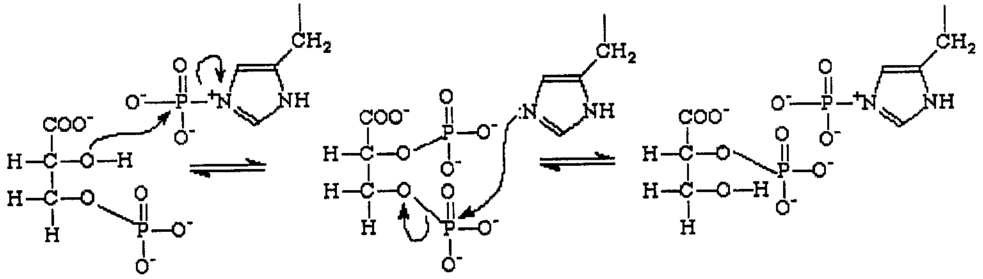
8) Phosphoglycerate Mutase: 3-PGA to 2-PGA, begins the **next stage** of Glycolysis and our ATP energy "profit."



This enzyme requires 2,3-*bis* PGA (2,3BPG; DPG) as a cofactor to phosphorylate the enzyme and to maintain the E-P intermediate:

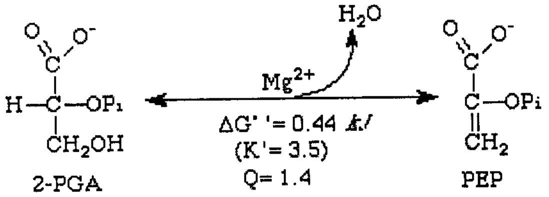
3-PGA + E-P $\rightleftharpoons$ 2,3-*bis*PGA + E $\rightleftharpoons$ 2-PGA + E-P

A detailed mechanism for this enzyme is shown below:

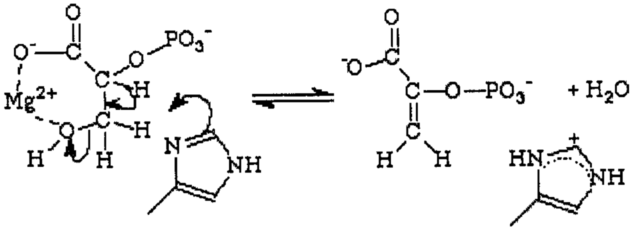

CH₂
O
O⁻—P—⁺N
NH
O⁻
COO⁻
H—C—O—H
H—C—O
H
O
P—O⁻
O⁻
COO⁻
O
P—O⁻
O⁻
:N
NH
H—C—O
H—C—O
H
O
P—O⁻
O⁻
CH₂
COO⁻
O
O⁻—P—⁺N
NH
O⁻
H—C—O
O
P—O⁻
H—C—O—H
O⁻
H

Note that there should be need for another enzyme to produce the BPG cofactor: Bisphosphoglycerate mutase- This enzyme catalyses the interconversion of 1,3-*bis* PGA to 2,3-*bis* PGA, taking a high energy compound to a low energy compound: this enzyme is thus an obvious candidate for control, since if it had much activity it could drain Glycolysis of ATP production! Normally of very low activity. (But enhanced in RBC's, since they use BPG to control the binding of oxygen by haemoglobin. RBC's also have another enzyme, 2,3-*bis* PGA Pase to bring BPG back into Glycolysis as 2-PGA, but without making ATP.)

9) Enolase: 2-PGA to PEP

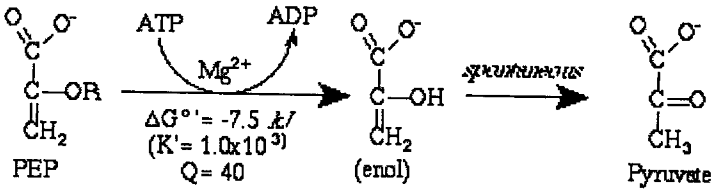

O
O⁻
C
H—C—OPi
CH₂OH
2-PGA
H₂O
Mg²⁺
ΔG' '= 0.44 kJ
(K'= 3.5)
Q= 1.4
O
O⁻
C
C—OPi
CH₂
PEP

This is an alcohol elimination reaction with catalysis by Magnesium and using general base catalysis by the enzyme:

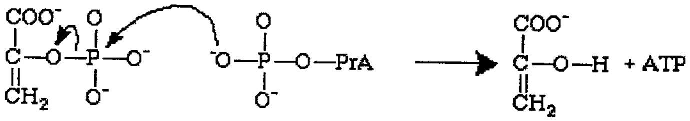

O
O⁻—C
PO₃⁻
O
C
H
Mg²⁺
O
C
H
H
H
N
NH
O
⁻O—C
C—O—PO₃⁻
+ H₂O
C
H
H
HN
+
NH

Note that a low energy compound (2-PGA, approx 10 kJ) is converted to a high energy compound (PEP, greater than 60 kJ) with very little change in energy overall. Essentially have made the phosphate bond much less stable, while increasing the stability of other bonds in the molecule.

10) Pyruvate Kinase: PEP to Pyruvate



Here, there is an attack by ADP:



The resulting enol then spontaneously tautomerizes to pyruvate.

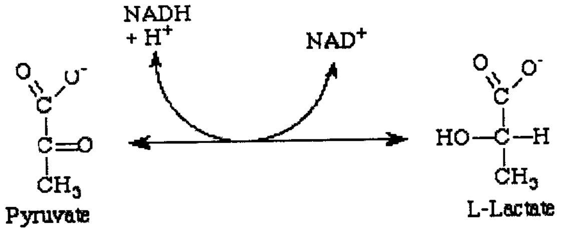
PK Isozymes

PK is a regulatory enzyme in some tissues. There are three isozymes:

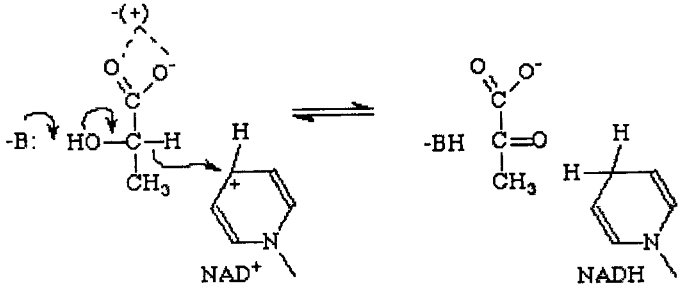
- L-PK (Liver, Kidney, RBC's): greatest regulation. Sigmoidal vs. [PEP] & [K^+]; F-1,6-*bis* P is a (+) effector to both. Hormonal control operates via phosphorylation to inactivate the enzyme.
- M-PK (Muscle, brain): least regulation - product inhibition by Pyruvate and MgATP.
- K-PK (Adipose, kidney, liver): intermediate regulation. Sigmoidal vs. [PEP] & [K^+]; F-1,6-*bis* P is a (+) effector to both.

PK completes the reactions of Glycolysis. However, for Glycolysis to proceed NAD^+ needs to be regenerated. For aerobic tissues this is done via the Kreb's TCA Cycle.

Lactate DH is used to regenerate NAD^+ in anaerobic tissue in mammals, and takes Pyruvate to Lactate:



Again the NAD+ abstracts a Hydride ion in the reverse reaction:



while a general base aids the formation of the carbonyl carbon, and a positive charge draws electron charge up to the carboxyl group and aids the removal of the hydride ion.

Lactate DH also has isozymes. It is a tetramer of two types of monomers, H & M. Can thus have 5 possible isomers: H_4, H_3M, H_2M_2, HM_3, & M_4, with one active site per monomer.

LD Isozymes

Lactate DH is a tetramer of two types of monomers, H & M. The kinetic properties of these pure monomer LD isozymes are given in the below Table: 9.2

Table 9.2 : Kinetic properties of LD Isozymes.

Michaelis Constant (K_M)		
	H	M
Pyruvate	1.4×10^{-4}	5.2×10^{-4}
Lactate	9×10^{-3}	2.5×10^{-2}
Pyruvate Inhibition?	yes	no

The properties of the H(eart) monomer, which predominates in aerobic tissues can be rationalized as better adapted to the aerobic environment. (Heart uses lactate from the serum as a fuel, but doesn't want to lose pyruvate produced in glycolysis to lactate production).

Regulation of Glycolysis

Regulation and Control: The concentration of citrate also affects PFK activity as a negative effector.

- **PFK is the Main regulatory enzyme** (flux generating step, first committed step) :
    - ATP is a strong negative effector of PFK.
    - Inhibition by "product" of pathway rather than enzyme, as expected for flux generating step.
        - AMP is a strong positive effector for PFK, overcoming ATP effects, used as a "proxy" for ATP energy levels because if changes much greater, thus "amplifying" changes in [ATP].
- **Hexokinase is regulated indirectly by PFK.**
    - Regulated by product inhibition by G-6-P.
        - G-6-P indicator of glucose use by the various pathways leading from it (Glycolysis, Glycogen synthesis/breakdown, Pentose Phosphate Pathway)
    - If PFK shuts down, then G-6-P builds up (if other uses also shut down), turning off HK.
    - If PFK active, then [G-6-P] decreases, allowing HK to resume activity.
- **Pyruvate Kinase is also regulated indirectly by PFK.** (**Note** there are other isozymes with differing regulation).
    - PK shows homotropic allosterism towards its substrate, PEP (sigmoidal kinetics).
    - PFK product, F-1,6-bisP, is a positive *feed-forward* activator of PK.
        - If PFK is active F-1,6-bisP builds up (free energies of glycolysis between F-6P and PEP favor).

Significance of Glycolysis

Glycolysis is an almost universal central pathway of glucose catabolism occurring in the cytoplasm of all the tissues of biological systems leading to generation of energy in the form of ATP for vital activities. It is the pathway through which the largest flux of carbon occurs in most cells. In plants, glycolysis is the key metabolic component of the respiratory process, which generates energy in the form of ATP in cells where photosynthesis is not taking place. Many types of anaerobic microorganisms are entirely dependent on glycolysis.

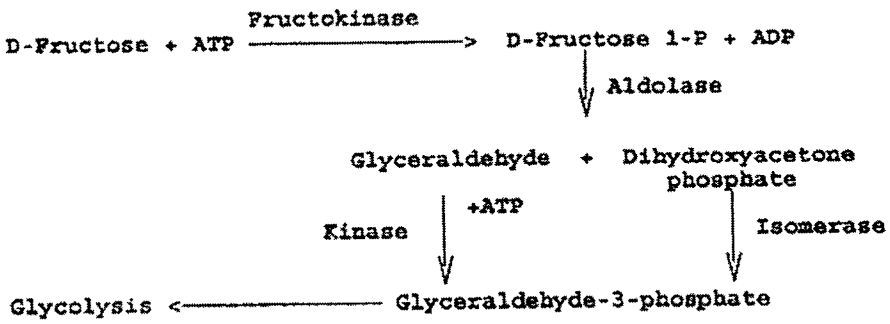
Mammalian tissues such as renal medulla and brain solely dependent on glycolysis for major sources of metabolic energy.

Energy Yield

Two ATPs are used in glycolysis and four ATPs are synthesized for each molecules of glucose so that the net yield is two ATPs per glucose. Under aerobic conditions, the two NADH molecules arising from glycolysis also yield energy via oxidative phosphorylation.

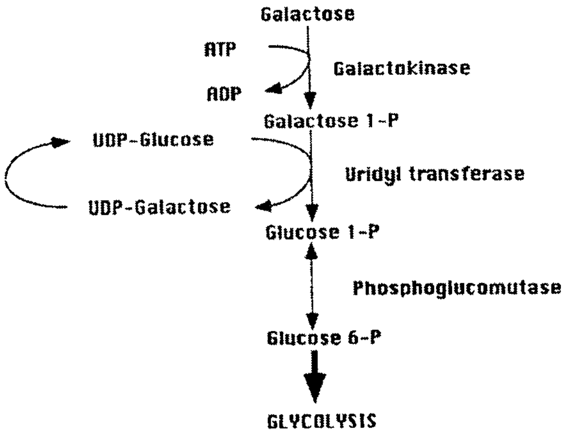
Fructose Metabolism:

Occurs in **liver**.


Fructokinase
D-Fructose + ATP
D-Fructose 1-P + ADP
Aldolase
Glyceraldehyde + Dihydroxyacetone phosphate
+ATP
Kinase
Isomerase
Glycolysis
Glyceraldehyde-3-phosphate

Galactose Metabolism:

Occurs in **liver**.


Galactose
ATP
ADP
Galactokinase
Galactose 1-P
UDP-Glucose
UDP-Galactose
Uridyl transferase
Glucose 1-P
Phosphoglucomutase
Glucose 6-P
GLYCOLYSIS

UDP-Glucose is an activated form of glucose found as an intermediate in **glycogen formation.**

UDP-Glucose is recycled from **UDP-Galactose** thus, there is **no NET change in concentration** of this compound.

Fructose and Galactose Energy Production: still 2 ATP.

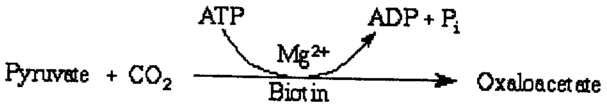
(Fructose enters at glyceraldehyde-3-phosphate)

(Galactose enters at glucose-6-phosphate)

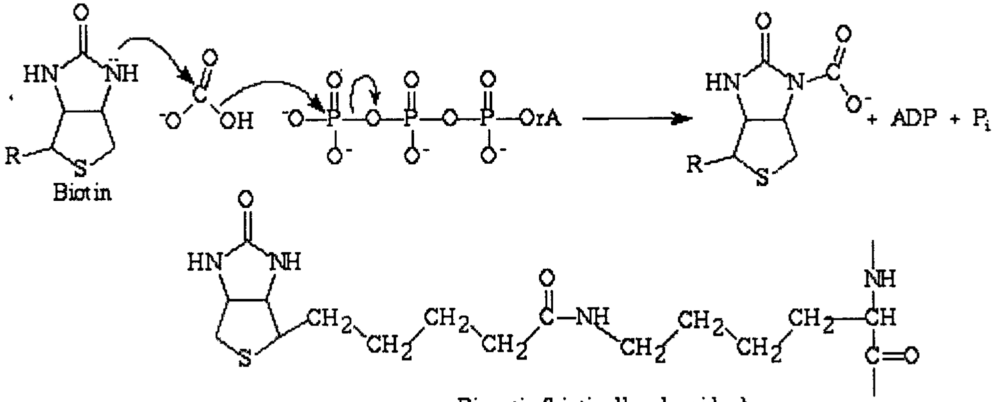
Gluconeogenesis

In order to provide glucose for vital functions such as the metabolism of RBC's and the CNS during periods of fasting (greater than about 8 hrs after food absorption in humans), the body needs a way to synthesis glucose from precursors such as pyruvate and amino acids. This process is referred to as gluconeogenesis. It occurs in the liver and in kidney. Most of Glycolysis can be used in this process since most glycolytic enzymes are operating at equilibrium. However three irreversible enzymes must be bypassed in gluconeogenesis vs. glycolysis: Hexokinase, Phosphofructokinase, and Pyruvate kinase. Phosphofructokinase, and/or hexokinase must also be bypassed in converting other hexoses to glucose.

Pyruvate converted to PEP without using the pyruvate kinase reaction? Formally, pyruvate is first converted to oxaloacetate, which is in turn converted to PEP. In the first reaction of this process **Pyruvate carboxylase** adds carbon dioxide to pyruvate with the expenditure of one ATP equivalent of energy. Biotin, a carboxyl-group transfer cofactor in animals, is required by this enzyme:

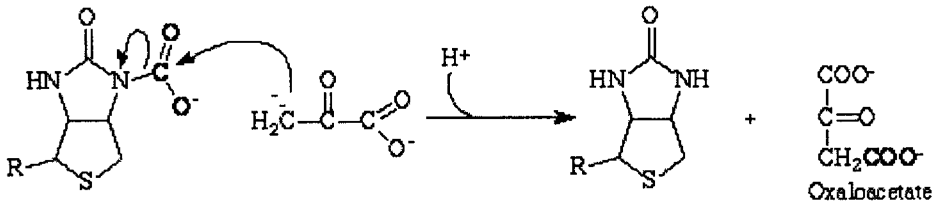


The reaction takes place in two parts on two different sub-sites on the enzyme. In the first part biotin attacks bicarbonate with a simultaneous attack/hydrolysis by bicarbonate on ATP, resulting in the release of ADP and inorganic phosphate (note the coupling by the enzyme of independent processes in this reaction):

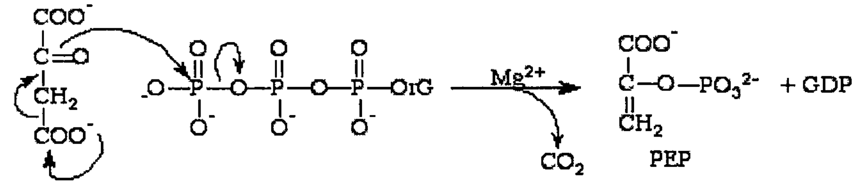


Biocytin (biotinyllysyl residue)

Note that the 14 Angstrom arm of biocytin allows biotin to move between the two sites, in this case carrying the activated carboxyl group. In the second site a pyruvate carbanion then attacks the activated carboxyl group, regenerating the biotin cofactor and releasing oxaloacetate:

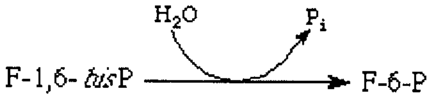


Pyruvate carboxylase is followed by the **Phosphoenolpyruvate carboxykinase** (PEPCK) reaction. In this reaction oxaloacetate is decarboxylated with a simultaneous phosphorylation by GTP to give GDP:

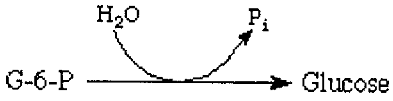


In eukaryotes the transformation of Pyruvate to Phosphoenol pyruvate (PEP) is further complicated by the fact that oxaloacetate is generated from pyruvate and TCA Cycle intermediates only in the mitochondria, while PEP is converted to glucose in the cytosol. And oxaloacetate cannot cross the mitochondrial membrane efficiently (it is present at concentrations way below the K_M of the carrier, so it must be converted into malate or aspartate in order to cross the membrane.

The glycolytic reactions from PEP to F-1,6-*bis*P are fully reversible, but a second bypass is required to get around PFK. This is accomplished by **Fructose-*bis*Phosphatase** (F-*bis*Pase):



And finally the third irreversible reaction, Glucokinase, is bypassed by **Glucose-6-Phosphatase** (G-6-Pase):



Note that by hydrolyzing off the phosphates in these two reactions rather than recovering ATP they are made energetically favourable. Thus both glycolysis and gluconeogenesis can be favourable processes, even though they proceed in opposite directions: For Glycolysis, free energy= about - 80 kJ, and for Gluconeogenesis, free energy= about -36 kJ due to differences in ATP. Or another way: if take glucose to pyruvate and then back to glucose again, 4 ATP's are lost.

In order to accomplish gluconeogenesis-reducing equivalents in the form of NADH must be provided to the GA-3-P DH enzyme and ATP must be provided to PGA Kinase. The obvious source for these is the Mitochondria, but then there are transport problems.

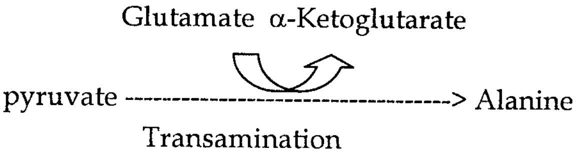
Precursors for Gluconeogenesis

1) Lactate

Cori cycle - no net gain or loss of glucose

Anaerobic respiration of pyruvate.

2) Amino Acids



3) Glycerol

$$\text{Glycerol} \xrightarrow{\textbf{Glycerol kinase}} \text{Glycerol 3-phosphate} \longrightarrow \text{DHAP}$$

If glycerol 3-phosphate dehydrogenase is embedded in inner mitochondrial membrane, e- passed to ubiquinone.

If enzyme is cytosolic, NADH is also a product.

Regulation of Gluconeogensis

- Glycolysis and gluconeogenesis are reciprocally regulated.
- If both pathways were activated, e.g.,

  Fructose 6-phosphate + ATP ------> Fructose 1,6-bisphosphate + ADP

  Fructose 1,6-bisphosphate + H_2O ---> Fructose 6-phosphate + P_i

  Net reaction: ATP + H_2O ---> ADP + P_i
- Called **substrate cycle** ---> "burn" 4 ATPs for every 2 ATPs made (can be used to generate heat).
- Reason why enzymes are regulated --> prevents this from happening.
- Two regulatory points are the two steps which had different enzymes.

  Fructose 1,6-bisphosphatase : inhibited by AMP and fructose 2,6-bisphosphate

  Pyruvate carboxylase : activated by acetyl CoA.

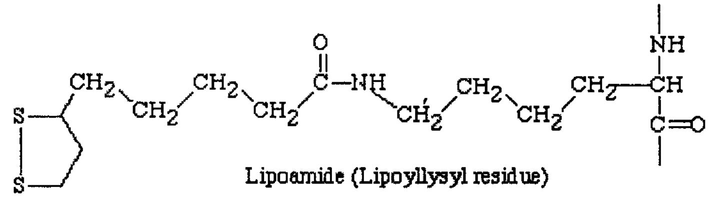
Pyruvate Metabolism

Let's turn now to the fate of pyruvate in aerobic tissues. Pyruvate must first be transported into the mitochondria, where it can then be oxidized to give acetyl CoA, which can then be used to make fat for storage or it can be further oxidized to carbon dioxide via the Kreb's TCA Cycle.

The oxidation of pyruvate to acetyl CoA is accomplished by the **Pyruvate Dehydrogenase complex**, a large, multi-component enzyme with three main enzyme subunits.

The first enzyme of this complex, **pyruvate dehydrogenase** (note that, unusual for the DH appellation, there is no direct NAD^+ or FAD involvement), catalyzes two sequential reactions. In the first reaction, catalyzed by the subunit of the enzyme, the coenzyme Thiamine Pyrophosphate (TPP), with a highly acidic carbon (a stable carbanion), attacks pyruvate at C-2 with the loss of carbon dioxide to give a covalent coenzyme-substrate intermediate. In the second reaction, catalyzed by the beta subunit, the ketol group is oxidatively transferred to one of the sulphurs of the lipoyl coenzyme on the second enzyme of the complex, **dihydrolipoyl transacetylase,** to give an acetyl-lipoamide intermediate.

The lipoamide of dihydrolipoyl transacetylase constitutes a long arm which may now move the acetyl group from the active site of pyruvate DH to its own active site where the lipoamide is exchanged for Coenzyme A-SH. (On the mammalian enzyme the 60 subunits of the transacetylase seem to form a pool of lipoyl groups among which the acetyl groups are freely exchanged).



Lipoamide (Lipoyllysyl residue)

Note that in the reactions of dihydrolipoyl transacetylase the lipoamide has been reduced from a disulphide to two sulphhydryl groups. In order to continue operation lipoamide must be reoxidized and that is accomplished by the final enzyme of the complex, **dihydrolipoyl dehydrogenase.** The reactions catalyzed by this enzyme are complex, but the net result is the transfer of two electrons from the lipoamide to NAD^+ to give NADH.

Overall then the **Pyruvate DH Complex** converts pyruvate into acetyl CoA in a **physiologically irreversible reaction** with the release of carbon dioxide and the capture of an electron pair as a hydride ion on NADH. Note the **cofactors** involved for this reaction sequence: **TPP, FAD, Mg^{2+}, lipoamide, Coenzyme A, and NAD^+.**

Conversion of Pyruvate to Acetyl COA

Enzyme is pyruvate dehydrogenase complex, composed of three enzymes:

1) Pyruvate dehydrogenase.
2) Dihydrolipoamide acetyltransferase.
3) Dihydrolipoamide dehydrogenase.

Reaction occurs in 5 steps:

- E_1 uses TPP as a prosthetic group and decarboxylates pyruvate --> forms HETPP intermediate.
- E_1 then transfers acetyl group to oxidized lipoamide --> acetyllipoamide.
- E_2 transfers acetyl group to coenzyme A to form acetyl CoA; dihydrolipoamide becomes reduced.
- E_3 reoxidizes lipoamide portion of E_2; prosthetic group of E_3 (FAD) oxidizes reduced lipoamide --> $FADH_2$.
- NAD^+ is reduced by E_3-FADH --> E_3-FAD + NADH + H^+.

E2 acts like a crane by swinging substrate between protein complexes in enzyme.

Regulation of PDH complex:

Regulated by covalent modification by phosphorylation.

Inactive = Phosphorylated; Active = Dephosphorylated

E_1 Inhibited at high [ATP]; inhibited at high [GTP]
Activated by high [AMP], high [Ca^{2+}], high [pyruvate]

E_2 Inhibited by high [acetyl CoA]
Activated by high [CoA-SH]

E_3 Inhibited by high [NADH]
Activated by high [NAD^+]

Kreb's TCA Cycle

The **Tricarboxylic acid** cycle is in many ways the central pathway of metabolism, both catabolically and anabolically: it is involved in the breakdown and synthesis of a variety of compounds. The oxidative breakdown of the acetyl group of acetyl CoA (Fig. 9.5).

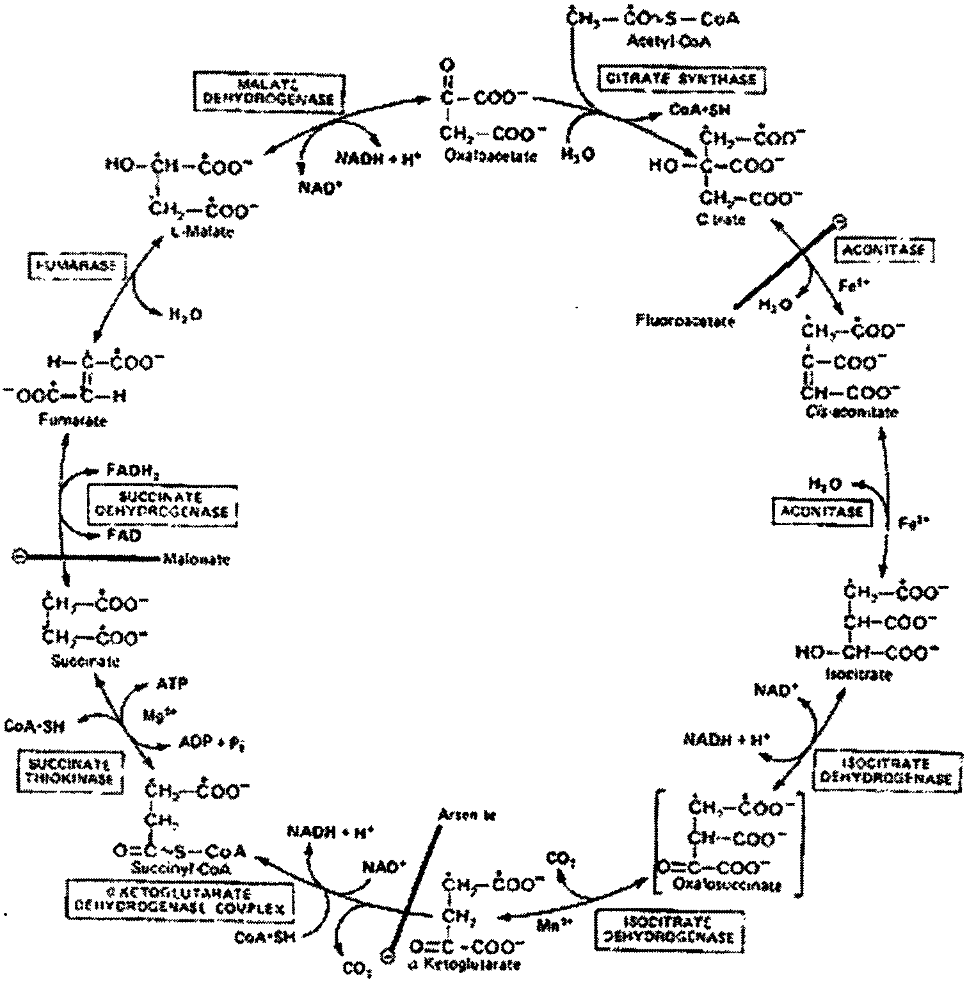
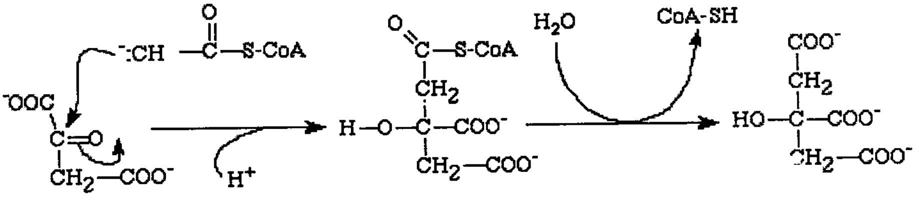

Acetyl-CoA
CITRATE SYNTHASE
CoA-SH
Oxaloacetate
MALATE DEHYDROGENASE
NADH + H+
NAD+
H2O
Citrate
ACONITASE
Fe2+
Fluoroacetate
Cis-aconitate
ACONITASE
Isocitrate
NAD+
NADH + H+
ISOCITRATE DEHYDROGENASE
Oxalosuccinate
ISOCITRATE DEHYDROGENASE
Mn2+
CO2
α Ketoglutarate
Arsenite
NADH + H+
NAD+
α KETOGLUTARATE DEHYDROGENASE COMPLEX
CoA-SH
CO2
Succinyl-CoA
SUCCINATE THIOKINASE
CoA-SH
ATP
Mg2+
ADP + Pi
Succinate
Malonate
FADH2
SUCCINATE DEHYDROGENASE
FAD
Fumarate
FUMARASE
H2O
L-Malate

Fig. 9.5 : Kreb's TCA Cycle.

First condense the acetyl group with a four carbon carrier to get a six carbon tri-acid. This is then rearranged and oxidized with loss of carbon dioxide to give a five carbon di-acid ketol very similar to pyruvate in structure. An **irreversible** DH Complex then creates a four carbon CoA derivative with the release of a second carbon dioxide. At this point it appears that acetyl has been released as carbon dioxide, however, the carrier has been reduced, and modified. A series of reactions now regenerates the original carrier.

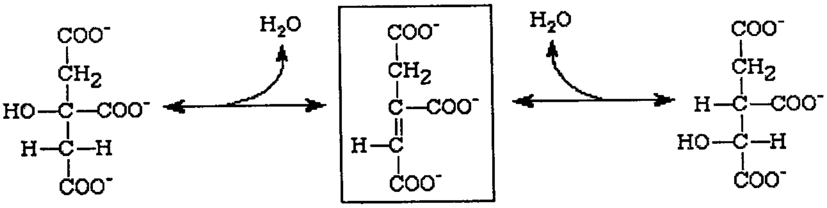
The first reaction of the cycle is an aldol condensation catalyzed by :

Citrate Synthase:



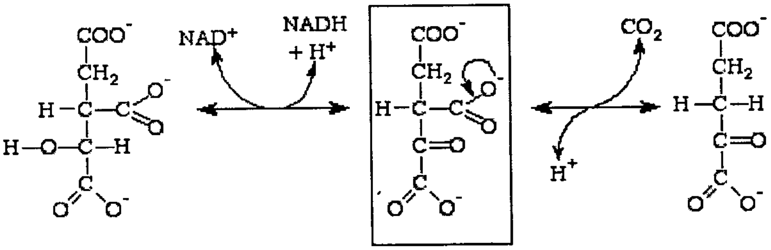
Note that the enzyme catalyst enables the coupling of two chemically independent reactions: the aldol condensation (with free energy change of about zero) to the very favourable hydrolysis of the CoA thiol ester bond which drives the overall reaction far towards product. Essentially, we have used an ATP's worth of energy to drive the reaction to completion.

Unfortunately the resulting citrate is a tertiary alcohol which cannot be readily oxidized. **Aconitase** catalyzes the rearrangement of citrate to give an oxidizable secondary alcohol. This reaction involves an elimination/addition sequence, catalyzed by an iron-sulphur cluster (Fe_4S_4), with an alkene intermediate, *cis*-Aconitate:



It is then converted the 3° alcohol, citrate, into an oxidizable 2° alcohol, isocitrate. The next reaction is the first oxidation of the TCA Cycle.

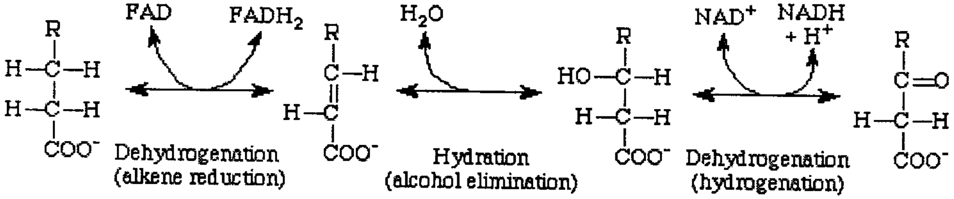
The isocitrate alcohol can now be oxidized with NAD^+ by **Isocitrate DH** to give an enzyme bound intermediate. The intermediate has a carboxyl group *beta* to a carbonyl carbon, so it has an excellent leaving group, CO_2, attached to a stabilized carbanion. Thus it immediately rearranges to lose carbon dioxide:



The resulting 2-oxo-glutarate (α-ketoglutarate) looks just like pyruvate with an R-group attached to the β-carbon, so it is broken down by a DH Complex, the **α-Ketoglutarate DH Complex**, just as pyruvate was. This gives succinyl-CoA and releases a second carbon dioxide. Note that at this point two carbons have been released, so formally, we have released the two carbons of Acetyl-CoA (Though neither of them came from the acetyl CoA we added)! We have also produced two NADH's (4 NADH/Glucose) which will result in the production of 5 ATP's (or: 4 x 2.5 ATP/NADH= 10 ATP's/Glucose). The remainder of the cycle is involved in the regeneration of oxaloacetate.

Succinyl-CoA, like acetyl-CoA, has a high-energy bond. However in this case the energy will be captured, using **Succinyl-CoA Synthetase**, to give a GTP which is energetically equivalent to an ATP (2 ATP's/Glucose). The mechanism of this reaction first involves the phosphorolysis by inorganic phosphate of the thiol ester bond to give a phosphoric-carboxylic mixed acid anhydride, followed by formation of a phosphorylated enzyme and finally transfer of the phosphate onto GDP.

The reactions beginning with succinate are representative of a common pattern, the "Mainline Sequence," seen repeatedly in biochemical pathways.


FAD
FADH₂
H₂O
NAD⁺
NADH + H⁺
R
H—C—H
H—C—H
COO⁻
Dehydrogenation (alkene reduction)
C—H
H—C
Hydration (alcohol elimination)
HO—C—H
Dehydrogenation (hydrogenation)
C=O

First, **Succinate DH**, an inner-mitochondrial membrane-bound enzyme and member of the mitochondrial electron transport system (ETS), oxidizes succinate to fumerate. This reaction uses the stronger oxidizer FAD as an oxidizing agent because of the added difficulty in oxidizing an alkane to an alkene. As a consequence of using this more powerful oxidizing agent, less ATP energy can be captured in oxidizing the resulting $FADH_2$ with oxygen (FAD is closer to oxygen in its oxidation potential). One and one-half ATP equivalents are obtained in this reaction (or: 2 x 1.5 ATP/$FADH_2$= 3 ATP/Glucose).

The resulting alkene, Fumerate, is not readily oxidized. However, if water is added across the double bond an alcohol results which can be oxidized. Thus **Fumerase** catalyses a hydration reaction to give malate.

Finally, **Malate DH** catalyzes the dehydrogenation of malate to regenerate the original carrier, oxaloacetate, and finish the cycle. In addition another NADH is formed (and 2 x 2.5 ATP/NADH= 5 ATP/Glucose).

For the entire cycle, the production of 10 ATP/acetyl-CoA or 20 ATP/Glucose. The aerobic catabolism of glucose can then give a maximum total of 32 ATP/glucose by TCA cycle as summarized in below Table. 9.3

Table 9.3 : Calculation of ATP yields upon complete Aerobic oxidation of Glucose.

Reaction	**Energy Product**	**Factor**	**ATP Equivalents** (@ 2.5 ATP/NAD)	**ATP Equivalents** (@3 ATP/NAD)
Glycolysis				
Hexokinase	ADP	1 x -1	- 1	-1
PFK	ADP	1 x-1	- 1	-1
GA-3-P DH	NADH	2 x 2.5 (1.5)*	5 (3)*	6 (4)*
PGA Kinase	ATP	2 x1	2	2
Pyruvate Kinase	ATP	2 x 1	2	2
Pyruvate DH Complex & Kreb's Cycle				
Pyruvate DH Complex	NADH	2 x 2.5	5	6
Isocitrate DH	NADH	2 x 2.5	5	6
2-oxoglutarate DH Complex	NADH	2 x 2.5	5	6
Succinyl-CoA Synthetase	GTP	2 x 1	2	2
Succinate DH	$FADH_2$	2 x 1.5	3	4
Malate DH	NADH	2 x 2.5	5	6
		TOTAL=	32 (30)*	38 (36)*
* In some tissues (insect flight muscle, fast twitch muscle) the reducing equivalents of NADH must be pumped against a gradient at a cost of 1 ATP (it is used to make $FADH_2$).				

Note that because all catalysts (oxaloacetate, enzymes etc.) must be regenerated in looking at the overall operation of the cycle, only the acetyl group of acetyl-CoA can be oxidized completely. Some intermediates, such as citrate, can be partially oxidized, but Kreb's cycle intermediate catabolism requires leaving the cycle at oxaloacetate and then returning as acetyl-CoA. It requires leaving the mitochondria for some reactions, and since the extremely low concentrations of oxaloacetate don't allow its efficient transport across the mitochondrial membrane (the K_m of the carrier is much higher than [oxaloacetate]), malate is the species which actually leaves the mitochondria.

Indeed :

- Composed of 8 reactions.
- 4 carbon intermediates are regenerated.
- 2 molecules of CO_2 released (6C--> 4C).
- Most of energy stored as NADH and QH_2.

Regulation of the TCA Cycle

In order to understand the regulation of the TCA cycle, it is necessary to look at the ΔG values for the various reactions and the kinetic properties of the enzymes. Values for the non-equilibrium reactions are tabulated below as Table 9.4:

Table 9.4 : Kinetics of TCA Cycle Regulatory Enzymes.

Enzyme	Substrate	Substrate Conc. (µM)	K_m (µM)	ΔG (kJ/mol)	Effectors
Citrate synthase	Acetyl-coa	100-600	5-10	-53.9	Succinyl CoA (-), ATP (-), NADH (-)
	Oxaloacetate	1-10	5-10		
Isocitrate DH (NAD^+)	Isocitrate	150-700	50-200	-17.5	Ca^{2+} (+), ATP (-), ADP(+), NADH (-)
2-Oxoglutarate DH	2-oxoglutarate	600-5900	60-200	-43.9	Ca^{2+} (+), Succinyl CoA (-), NADH (-)
Data from E. A. Newsholme and A. R. Leach Biochemistry for the Medical Sciences. John Wiley & Sons, New York (1983) pp 101 & 110.					

Normally the concentrations of ATP, ADP, NAD^+, and NADH are relatively constant in the mitosol and are thus unlikely to be very effective as allosteric regulators under most circumstances. On the other hand the **availability** of NAD^+ and FAD **as substrate** will affect the rate not only of the reactions in the table, but also the near-equilibrium dehydrogenases. NAD^+ availability in turn is determined by the activity of the electron transport system, whose activity is closely coupled to the availability of ADP. Thus high [ATP] will slow the TCA cycle since high [ATP] means low [ADP], which will slow the ETS resulting in low [NAD^+]!

In muscle, Ca^{2+} does show significant changes in concentration in the mitosol (recall that an increase in [Ca^{2+}] concentration initiates muscle concentraction). Succinyl CoA will also show significant concentration changes under differing conditions and can thus also serve as an effective regulator, indicating carbon status in the second half of the cycle.

There are 2 enzymes that are regulated:

1) Isocitrate dehydrogenase

Allosterically activated by high [Ca^{2+}] and high [ADP]

Allosterically inhibited by high [NADH]

2) α-Ketoglutarate dehydrogenase

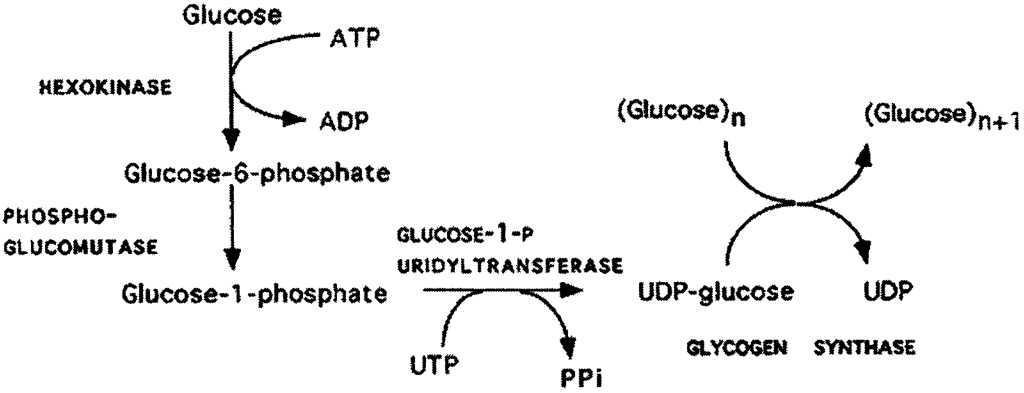
Allosterically activated by high [Ca^{2+}]

Allosterically inhibited by high [NADH] and high [succinyl CoA]

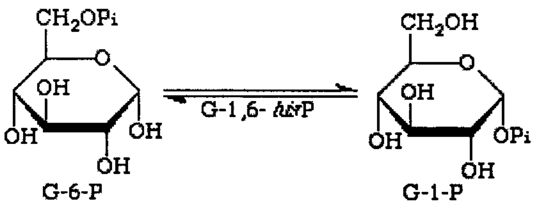
Interconversion of Metabolic Intermediates: The TCA cycle has a central place in metabolism (even in anaerobic organisms) via its use to interconvert metabolites.

Glycogen Metabolism

Glycogenesis:


Glucose
ATP
HEXOKINASE
ADP
Glucose-6-phosphate
PHOSPHO-
GLUCOMUTASE
Glucose-1-phosphate
GLUCOSE-1-P
URIDYLTRANSFERASE
UTP
PPi
UDP-glucose
(Glucose)n
(Glucose)n+1
UDP
GLYCOGEN SYNTHASE

Start with G-6-P, again note that this molecule is at a metabolic crossroads. First convert to G-1-P using **Phosphoglucomutase**:

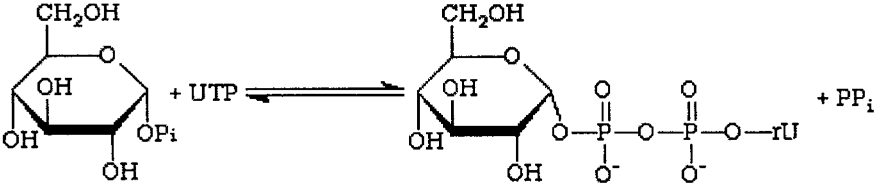

CH2OPi
O
OH
OH
OH
OH
G-6-P
G-1,6-[illegible]P
CH2OH
O
OH
OH
OPi
OH
G-1-P

This reaction is very much like PGA Mutase, requiring the *bis* phosphorylated intermediate to form and to regenerate the phosphorylated enzyme intermediate. Note that this reaction is easily reversible, though it favors G-6-P.

Again a separate "support" enzyme, Phosphoglucokinase, is required to form the intermediate, this time using ATP as the energy source:

$$\text{G-6-P} + \text{ATP} \underset{Mg^{2+}}{\rightleftharpoons} \text{G-1,6-bisP} + \text{ADP}$$

UDP-glucose pyrophosphorylase, which catalyzes the next reaction, has a near zero $\Delta G^{\circ\,\prime}$:



G-1-P

UDPGlucose

It is driven to completion by the hydrolysis of the PP_i to 2 P_i by **Pyrophosphatase** with a $\Delta G^{\circ\,\prime}$ of about -32 kJ (approx. one ATP's worth of energy).

Finally glycogen is synthesized with **Glycogen Synthase**:

$\text{UDPGlucose} + (\text{glucose})_n \longrightarrow \text{UDP} + (\text{glucose})_{n+1}$

This reaction is favoured by a $\Delta G^{\circ\,\prime}$ of about 12 kJ, thus the overall synthesis of glycogen from G-1-P is favoured by a standard free energy of about 40 kJ. Note that the glucose is added to the non-reducing end of a glycogen strand, and that there is a net investment of 2 ATP equivalents per glucose (ATP to ADP and UTP to UDP, regenerated with ATP to ADP). Note also that glycogen synthase requires a 'primer.' That is it needs to have a glycogen chain to add on to. What happens then in new cells to make new glycogen granules? Can use a special primer protein (glycogenin). Thus glycogen granules have a protein core.

These reactions will give linear glycogen strands, additional reactions are required to produce branching. **Branching enzyme** [amylo--(1,4) to -(1,6)-transglycosylase] transfers a block of residues from the end of one chain to another chain making a 1,6-linkage (cannot be closer than 4 residues to a previous branch). [*FYI:* For efficient release of glucose residues it has been determined that the optimum branching pattern is a new branch every 13 residues, with two branchs per strand].

Regulation of Glycogen Synthase:

Active - dephosphorylated
Inactive - phosphorylated

Glycogen Synthase-i: *independent* (i) of glucose-6-phosphate for its activity.

Glycogen Synthase-d: *dependence* (d) on *glucose-6-phosphate,* mechanism for storing glucose when overabundance is signalled by a build-up of glucose-6-phosphate (Fig. 9.6).

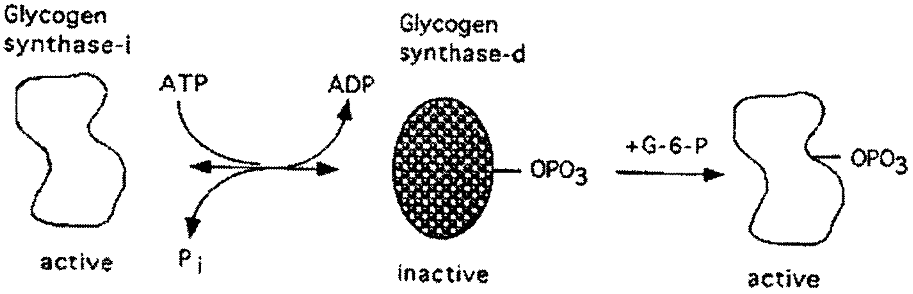

Glycogen synthase-i
ATP
ADP
Glycogen synthase-d
active
P_i
OPO_3
+G-6-P
OPO_3
inactive
active

Fig. 9.6 : Regulation of Glycogen Synthase

Glycogen (Fig. 9.7)

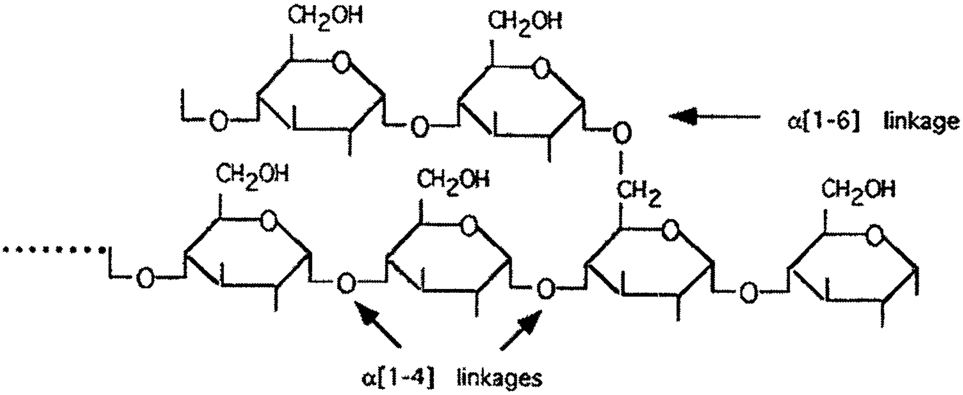

CH_2OH
CH_2OH
α[1-6] linkage
CH_2OH
CH_2OH
CH_2
CH_2OH
α[1-4] linkages

Fig. 9.7 : Structure of Glycogen

Glycogenolysis (Fig. 9.8)

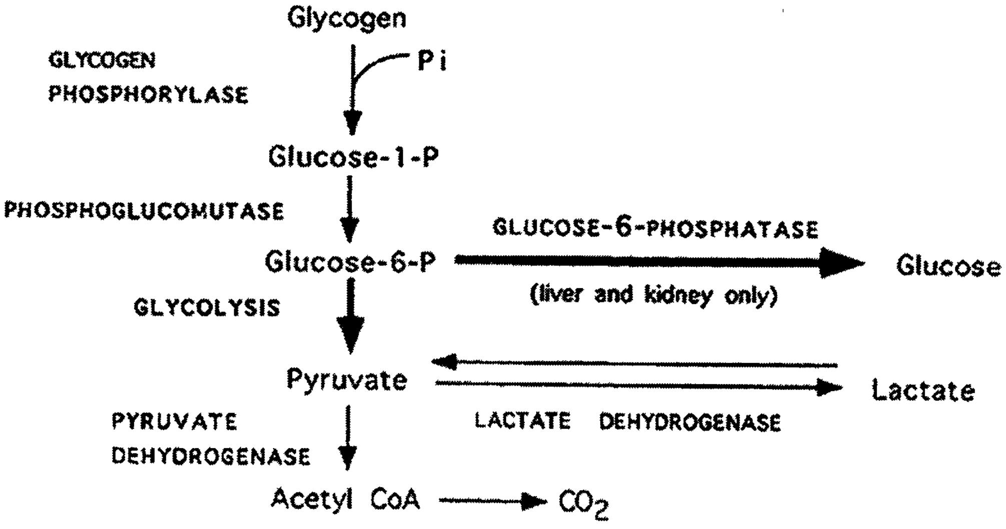

Glycogen
GLYCOGEN PHOSPHORYLASE
Pi
Glucose-1-P
PHOSPHOGLUCOMUTASE
Glucose-6-P
GLUCOSE-6-PHOSPHATASE
(liver and kidney only)
Glucose
GLYCOLYSIS
Pyruvate
Lactate
LACTATE DEHYDROGENASE
PYRUVATE DEHYDROGENASE
Acetyl CoA
CO_2

Fig. 9.8 : Glycogenolysis pathway

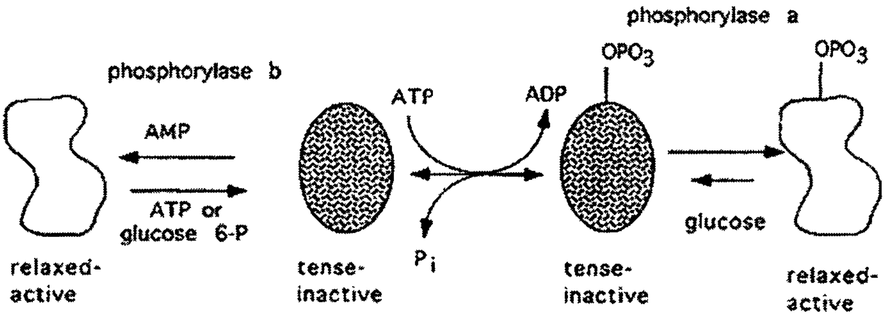
Glycogen is broken down using **Phosphorylase** to phosphorylize off glucose residues:

$$(\text{Glucose})_n + P_i \longrightarrow (\text{Glucose})_{n-1} + \text{G-1-P}$$

Note that no ATP is required to recover Glucose phosphate from glycogen. This is a major advantage in anaerobic tissues, get one more ATP/glucose (3 instead of 2!).

Phosphorylase can only cleave 1,4-linkages, so now need **Debranching enzyme.** Debranching enzyme has two activities: a) amylo--1,4-transferase moves the terminal three residues of a chain onto another branch; whereas -1,6-glucosidase hydrolyzes the 1,6-linkage to give free glucose. Thus, muscle can release a small quantity of glucose into the blood without actually doing gluconeogenesis.

Regulation: *complex,* to ensure glucose remains stored as glycogen until absolutely required to maintain blood glucose homeostasis or to supply energy to the cell.



- **Whether or not it is phosphorylated** it can exist in a "**tense**"(inactive) form or a "**relaxed**" (active) form (only **high glucose** can **force the existence of the phosphorylated tense** form).
- The enzyme can be **rapidly activated** without undergoing phosphorylation in response to a **hormonal signal.**

Activator: AMP

Inhibitor: ATP, glucose, glucose-6-phosphate

Glycogen Control

Since the Glycogen synthase and phosphorylase reactions are in opposition we need a control system. Glycogen storage/release strategies vary widely with different tissues. In liver, glycogen is used to provide glucose to the serum between meals - it serves a homeostatic function. Glycogen control in liver is thus designed to breakdown and release glycogen when serum [glucose] is low and synthesize glycogen when serum [glucose] is high.

Glycogen Control in Liver

In the liver glycogen metabolism is largely regulated by glucose concentrations, which in turn reflect serum glucose concentrations.

1. In liver **glycogen phosphorylase** *a* binds tightly to **protein phosphatase-1** and inhibits it. At high concentrations, glucose binds to phosphorylase, causing a release of the protein phosphatase. Protein phosphatase then inactivates *m*-phosphorylase *a* by hydrolyzing off P_i to give inactive *o*-phosphorylase *b*.
   - Glycogen breakdown and glucose release are inhibited.

2. Protein phosphatase can now also hydrolyze P_i from inactive **Glycogen synthase *b*** to give the active **Glycogen synthase *a*.**
    - Glycogen synthesis is activated.
3. As glucose concentrations drop, glycogen phosphorylase rebinds protein phosphatase and protein kinases rephosphorylate the enzymes, resulting in glucose release.

The net result is that glycogen is synthesized when [glucose] is high, and it is broken down when [glucose] is low.

Glycogen Control Cascade

In muscle it turns out that glycogen synthesis/breakdown is controlled by a very complex system enabling both rapid response to emergencies and exquisite overall control of the opposing activities to respond to a variety of situations. This is accomplished through the Glycogen Cascade Control system.

Response begins with a hormonal signal, such as adrenalin, binding to the receptor on the cell surface. This results in the phosphorylation of GDP to GTP on an intracellular G-protein. The G-protein can now interact with **Adenylate cyclase** to produce the "second messenger" 3', 5'- cyclic AMP (cAMP). Cyclic AMP then binds to the regulatory subunit of **cAMP-dependent protein kinase,** releasing the active catalytic subunits (C), which can now phosphorylate inactive ***o*-phosphorylase kinase *b*** to the active ***m*-phosphorylase kinase *a*** (o= original, m= modified, b= inactive, a= active). Phosphorylase kinase *a* then phosphorylates ***o*-Glycogen phosphorylase *b*** to the active ***m*-Glycogen phosphorylase *a*,** resulting in the breakdown of glycogen with the release of G-1-P.

Note the parallel kinase cascade which simultaneously shuts down **Glycogen synthase.**

Finally, one does not always have a warning, that is time to get the endocrine system going to produce adrenalin, thus the release of **Calcium** in the muscle cells bypasses much of the cascade, activating the normally inactive ***o*-Phosphorylase kinase *b*** , which then acts on both the phosphorylase and the synthase.

Pentose Phosphate Pathway (HMP Pathway)

- Provides NADPH (serves as e- donor) and forms ribose 5-phosphate (nucleotide synthesis).

- Pathway active is tissues that synthesize fatty acids or sterols because large amounts of NADPH needed.
- In muscle and brain, little PPP activity.
- All reactions are cytosolic.
- Divided into 2 stages:

The Pentose Phosphate Pathway is an alternate pathway for glucose oxidation which is used to provide reducing equivalents in support of biosynthesis. Thus although it involves the catabolism of glucose, it is generally going to be active only when anabolism is taking place (Fig. 9.9).

This pathway is usually treated in two parts: the *oxidative* portion, and the *sugar interconversions* portion. In the oxidative part, glucose is first oxidized to a lactone, and then oxidatively decarboxylated. Note that in each case $NADP^+$ is the oxidant as opposed to NAD^+. Note also that the two DH reactions are both physiologically irreversible, due in part to the very low concentrations of NADPH in cells.

The first enzyme, **G-6-P DH**, is highly specific for glucose (it is frequently used as the basis of specific glucose assays). In this reaction the #1 (aldehydic) carbon of glucose is oxidized to a lactone (cyclized carboxylic acid). This is the first committed step for this pathway and it is regulated by the availability of $NADP^+$ (substrate availability). Since $NADP^+$ and NADPH are in very low concentrations and the $NADP^+$/NADPH ratio is very low, and since $NADP^+$ is generated only during biosynthetic reactions this results in a close coupling of the oxidative portion of this pathway to reductive biosynthesis.

Next **Gluconolactonase** opens the ring with the addition of a molecule of water. Then 6-P-gluconate DH oxidizes the #3 carbon to a ketone. This results in the #2 carbon becoming somewhat acidic, thus destabilizing the carboxyl group, which is then lost to give the five carbon ribulose-5-P.

In the non-oxidative portion of the Pentose Phosphate Pathway a series of sugar interconversions takes the RU-5-P to intermediates of other pathways: Ribose-5-P for nucleotide biosynthesis, and F-6-P and Ga-3-P for glycolysis/ gluconeogenesis. All of these reactions are near equilibrium, with fluxes driven by supply and use of the three intermediates listed above.

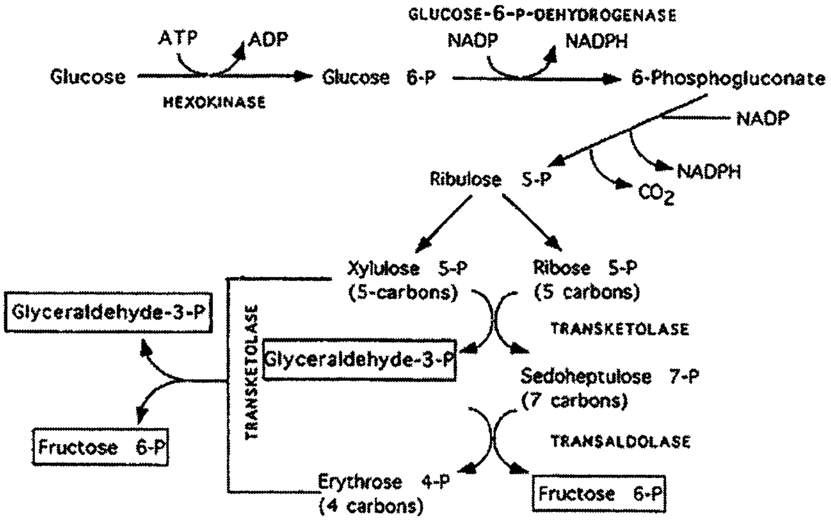
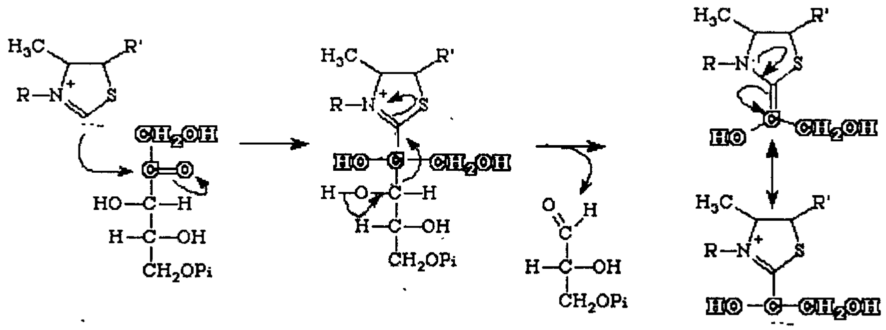

GLUCOSE-6-P-DEHYDROGENASE
ATP
ADP
Glucose
HEXOKINASE
Glucose 6-P
NADP
NADPH
6-Phosphogluconate
NADP
NADPH
CO_2
Ribulose 5-P
Xylulose 5-P
(5-carbons)
Ribose 5-P
(5 carbons)
TRANSKETOLASE
Glyceraldehyde-3-P
Glyceraldehyde-3-P
Sedoheptulose 7-P
(7 carbons)
TRANSALDOLASE
TRANSKETOLASE
Fructose 6-P
Erythrose 4-P
(4 carbons)
Fructose 6-P

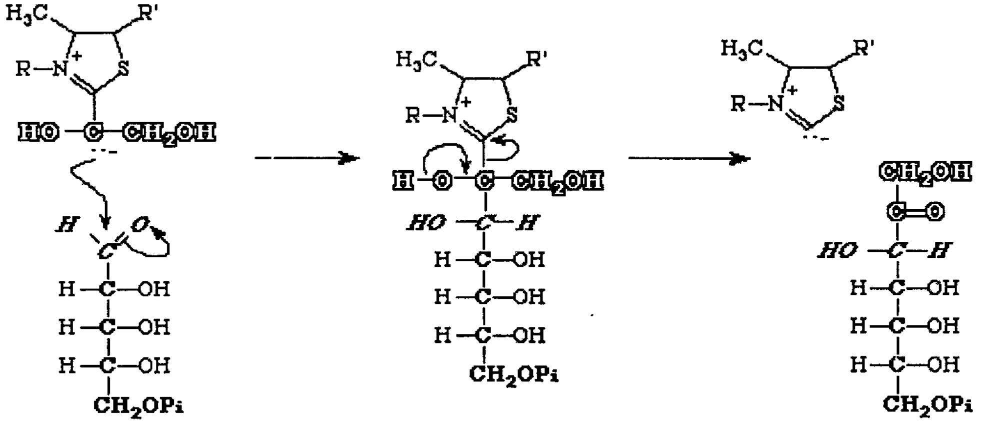
Fig. 9.9 : Schematic Presentation of Pentose Phosphate Pathway

In the first two reactions of this phase Ribulose-5-phosphate is converted either to Ribose-5-P via a 1,2-enediol intermediate, or to Xylulose-5-P via a 2,3-enediol intermediate.

These two 5-C sugars, R-5-P and Xu-5-P, are now interconverted to a 7-C sugar, Sedoheptulose-7-P, and a 3-C sugar, Glyceraldehyde-3-P. This reaction is catalyzed by **Transketolase**, a Thiamine pyrophosphate dependent enzyme which catalyzes the transfer of C_2 units. In the first part of this reaction the TPP carbanion makes a nucleophilic attack on the carbonyl group of xylulose. In the resulting intermediate the C2-C3 bond is destabilized and cleavage takes place to yield the enzyme bound 2-(1,2-dihydroxyethyl)-TPP resonance stabilized carbanion:



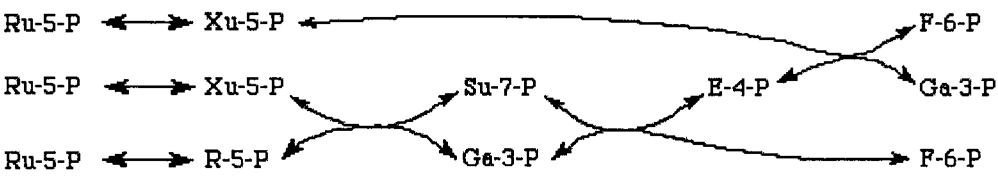
This first part of the reaction is very similar to the first part of the Pyruvate DH catalyzed reaction in the Pyruvate DH Complex. (Ga-3-P is the leaving group instead of carbon dioxide; there is a 1,2-dihydroxyethyl instead of a 1-hydroxyethyl carbanion intermediate.) In the second part of the reaction the carbanion then attacks the aldehyde of R-5-P to give Su-7-P and regenerate the TPP catalyst:


H3C
R'
R—N+
S
HO—C—CH2OH
H
O
C
H—C—OH
H—C—OH
H—C—OH
CH2OPi
H3C
R'
R—N+
S
H—O—C—CH2OH
HO—C—H
H—C—OH
H—C—OH
H—C—OH
CH2OPi
H3C
R'
R—N+
S
CH2OH
C═O
HO—C—H
H—C—OH
H—C—OH
H—C—OH
CH2OPi

This is similar to the second part of the Pyruvate DH reaction where the hydroxyethyl group attacks the disufide of the lipoamide.

Transaldolase catalyzes the transfer of a C3 unit. The reaction occurs via an aldol cleavage similar to that seen with aldolase: there is a schiff base intermediate formed with an active site lysine. The difference between aldolase and transaldolase is in the acceptor groups: in aldolase the acceptor is a proton, in transaldolase it is another sugar. This reaction yields a F-6-P, which can go to Glycolysis, and an E-4-P which reacts with Xu-5-P catalyzed by the same transketolase seen above. This second transketolase reaction yields F-6-P and Ga-3-P, both intermediates of Glycolysis and the end products of the Pentose-P pathway.

The interconversions of the sugars in this pathway are summarized in the flow diagram below:


Ru-5-P
Xu-5-P
F-6-P
Ru-5-P
Xu-5-P
Su-7-P
E-4-P
Ga-3-P
Ru-5-P
R-5-P
Ga-3-P
F-6-P

The principle products of this pathway are R-5-P and NADPH. Under reductive biosynthetic conditions where R-5-P is not needed the Pentose-P pathway can be used to completely oxidize G-6-P to 6 carbon dioxide molecules with the concomitant production of 12 NADPH's. Note also that when R-5-P is needed and NADPH is not needed for reductive biosynthesis it can be made from F-6-P and Ga-3-P.

The core reactions of the pathway can be summarized as -

$$\text{Glu-6-Phosphate} + 2\text{NADP}^+ + H_2O \longrightarrow \text{Ribose-5-Phosphate} + 2\text{NADPH} + H^+ + CO_2$$

Regulation of Pentose Phosphate Pathway

- Controlled by levels of NADP^+.
- Controlled step is dehydrogenation of glucose 6-phosphate to 6-phosphogluconolactone.
- Enzyme stimulated by high $[\text{NADP}^+]$.
- Nonoxidative branch controlled primarily by substrate availability.

Mitochondrial Electron Transport System

The electron transport system involves a variety of redox reactions, so it is useful to review some electrochemical relationships. First, the free energy of a reaction is related to the reduction potential for electron transfer by the equation:

$$\Delta G^{\circ\prime} = - nF\Delta E^{\circ\prime}$$

where n= moles of electrons transferred, and F is Faraday's constant (96,485 J $V^{-1}mol^{-1}$). The standard reduction potential for a reaction can be found from the difference between the reduction potentials of the electron acceptors and donors:

$$\Delta E^{\circ\prime} = \Delta E^{\circ\prime}_{e^- \, acceptor} - \Delta E^{\circ\prime}_{e^- \, donor}$$

The Nernst equation, which describes the reduction potential for an electrochemical reaction,

$$\Delta E = \Delta E^{\circ\prime} - (RT/nF)\ln Q$$

is very similar to the free energy equation,

$$\Delta G = \Delta G^{\circ\prime} + RT\ln Q,$$

while the equation for the reduction potential for an equilibrium system,

$$\Delta E^{\circ\prime} = (RT/nF)\ln K_{eq},$$

reminds one of the free energy/equilibrium relationship,

$$\Delta G^{\circ\prime} = - RT\ln K_{eq}.$$

Note the standard reduction potentials and resultant standard free energies: NADH: -0.315 V; O_2: +0.815 V. So for the reaction:

$$1/2\ O_2 + NADH \longrightarrow H_2O + NAD^+$$

Thus, 0.815 - (- 0.315) = 1.130 V, which, using the relationship between free energy and potential gives -218 kJ/mol. The free energy of hydrolysis of ATP is -30.5 kJ/mol, so if three ATP are made/pair of electrons flowing through ETS 91.5 kJ are captured out of 218 kJ available, or 42% . This is of course under "Standard Conditions." However, under physiological conditions this may actually be closer to 70%.

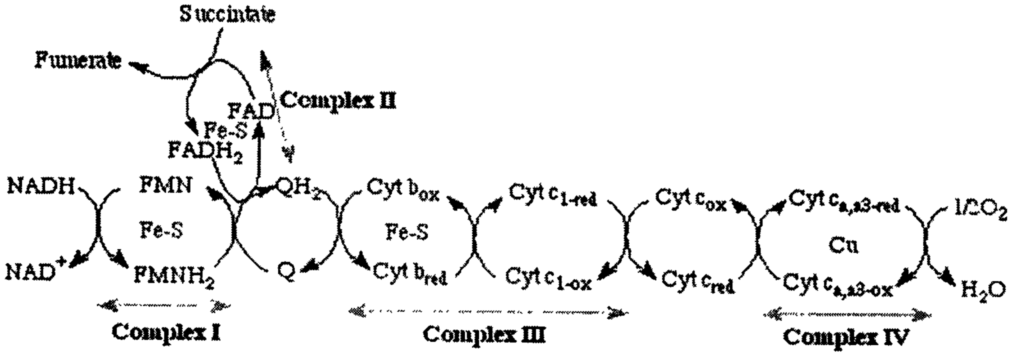
The Electron Transport System

The inner mitochondrial membrane is protein rich. If carefully broken down, it is very rich in five protein complexes: I -IV are large protein complexes involved in electron transport, while V is the ATP sythatase driven by proton gradients.

Electron flow through the ETS is summarized below. Only major electron carriers are shown within complexes.

NADH $\longrightarrow$[FMN $\longrightarrow$Fe-S] $\longrightarrow$Q $\longrightarrow$[Fe-S/Cyt *b* $\longrightarrow$Cyt c_1] $\longrightarrow$Cyt *c* $\longrightarrow$ □Cyt *a* $\longrightarrow$Cyt a_3] $\longrightarrow$$O_2$

Diagramatically:


Succintate
Fumerate
FAD
Complex II
Fe-S
FADH2
NADH
FMN
Fe-S
NAD+
FMNH2
QH2
Q
Cyt box
Fe-S
Cyt bred
Cyt c1-red
Cyt c1-ox
Cyt cox
Cyt cred
Cyt ca,a3-red
Cu
Cyt ca,a3-ox
1/2O2
H2O
Complex I
Complex III
Complex IV

Notice the central position of Coenzyme Q (aka ubiquinone, CoQ_{10}). The pool of CoQ forms a reservoir where reducing equivalents may be stored between the various inputs to the ETS, such as Complex I or Complex II and Complex III.

Succinate $\longrightarrow$[FAD $\longrightarrow$Fe-S] $\longrightarrow$Q

Glycerol-P $\longrightarrow$[FAD $\longrightarrow$Fe-S] $\longrightarrow$Q

Fatty acyl-CoA $\longrightarrow$Fatty acyl-CoA $DH_{soluble}$ $\longrightarrow$[FAD $\longrightarrow$Fe-S] $\longrightarrow$Q

With these electron transport components, path can be created for the transfer of electrons from substrate to oxygen. Complexes I and IV obviously have enough energy change to support the phosphorylation of ADP to ATP, while complex III is marginal and complex II obviously does not have sufficient energy change.

But how is this energy captured? Mitochondria appear to be using the energy of moving the electrons through this potential gradient to pump protons across the inner mitochondrial membrane. The resulting proton motive gradient can then be used to make ATP. Lets look at the four complexes and what seems to be occurring in each.

Complex I: The electrons are received as a pair (a hydride ion) on NADH, but most carriers can only handle single electrons so FMN acts as a transducer, picking up a pair of electrons, but passing them on singly to the FeS centers in this complex. The electrons are then passed singly on to CoQ, which, like FMN can carry either pairs or single electrons. Four H^+ are pumped across the inner mitochondrial membrane by complex I. (Note that formally the protons from NADH and H^+ can be considered as going to $FMNH_2$ and the UQH_2.).

Complex II: An electron pair enters this complex via FAD going to $FADH_2$. Of course FAD has the same active portion as FMN so it can again carry electrons single or in pairs. $FADH_2$ then passes its electrons on singly to FeS centers, which then pass them on to CoQ. No protons are pumped by this complex. Thus difference in the number of ATPs produced by NADH vs. $FADH_2$ can be distinguished.

Coenzyme Q (ubiquinone): CoQ is a quinone with a long hydrocarbon tail. It exists as a pool of CoQ molecules dissolved in and diffusing through the lipid bilayer of the inner membrane. Both the quinone (oxidized) and quinol (reduced) form of the cofactor can "flip" in the membrane, thus the quinone ring can freely cross. CoQ can pick up electrons one at a time from either Complex I or II, then diffuse through the bilayer until collision with complex III allows it to pass them singly to that complex.

Complex III: This complex picks up single electrons from the CoQ pool, taking the quinol form in two steps to the quinone form of the coenzyme. One electron is passed on through FeS centers to Cytochrome *c*, while a second is recycled via cytochrome *b* (b_{566} & b_{560}) to reduce Q to Q- in the Q-cycle. A second QH_2 repeats the cycle and provides the second electron for the first recycled Q. The net result is the oxidation of one QH_2 and the transport of four H^+ for each pair of electrons flowing through the complex.

Cytochrome C: This cytochrome is a mobile protein carrier attached to the outer side of the inner mitochondrial membrane (a peripheral membrane protein). It transports single electrons from complex III to complex IV.

Complex IV: This complex picks up electrons singly from cytochrome c and transfers them via cytochrome a-Cu_A to cytochrome a_3-Cu_B where they are passed on to oxygen. Note that four electrons are needed for each oxygen molecule, and that the equivalent of two H^+ are transferred out of the matrix for each pair of electrons (4 protons/oxygen). The high ΔG for this complex, which would allow a significantly larger number of protons to be pumped, assures the completion of the overall reaction of oxygen and NADH to give water.

(For an insider's review and evidence on ETS and OxPhos see M. Saraste *Science* **283** (5 March 1999) pp. 1488-93).

Oxidative Phosphorylation

Oxidative phosphorylation is the process in which ATP molecules are formed as a result of the transfer of electrons from the reducing equivalents, NADH or $FADH_2$ (produced by glycolysis, the citric acid cycle and fatty acid oxidation) to oxygen by a series of electron carriers in the form of a chain located in the inner membrane of mitochondria. This is the final reaction sequence of respiration. Since the electrons are transferred by a series of electron carriers in the form of a chain, it is known as electron transport system (ETS).

The electrons are transferred along a set of cytochromes in the form of a chain in steps from the more electronegative components ($NADH/FADH_2$) to the more electropositive oxygen.

ATP Synthase

ATP synthase uses the proton gradient to make ATP from ADP and P_i. It is bound to the inner membrane and has a characteristic knob and stalk structure. It can be broken into two multi protein components: The F_1 component (the "knob") hydrolyses ATP when it is isolated by itself and is referred to as F_1 ATPase. The F_0 component is a membrane spanning proton channel. When the two components are linked the passage of protons through the channel is coupled to ATP synthesis. According to the binding-change mechanism there are three sites in the $_3$beta$_3$ oligomer of the knob. At any given time the three sites are in three different conformations - open, loose, or tight. Each site passes sequentially through the three conformations, apparently while physically rotating 120° for each change. Following one site:

1) ADP and P_i bind to the site in the open conformation.

2) Passage of 3 protons through the channel causes the *-beta* oligomer to rotate 120° and change to the loose or L conformation, holding the ADP and P_i (all three active sites to go to the next conformation simultaneously).

3) Passage of another 3 protons through the channel causes the *-beta* oligomer to rotate 120° and change to the tight conformation with consequent condensation of ADP and P_i to ATP.

4) Passage of another 3 protons through the channel causes the *-beta* oligomer to rotate 120° and change to the open conformation, releasing ATP. Note the net result of 3 protons/ATP.

(For review and evidence on ETS and OxPhos see M. Saraste *Science* **283** (5 March 1999) pp 1488-93; for ATPase 'motor' see Paul D. Boyer (18 Nov 1999) "What matkes ATP synthase spin?" *Nature* 402: 247-8.)

Mitochondrial Transport/Communication

Given the requirement for a very tight inner-mitochondrial membrane in order to maintain the proton electrochemical gradient, how do important charged molecules such as ATP and NADH get across the membrane?

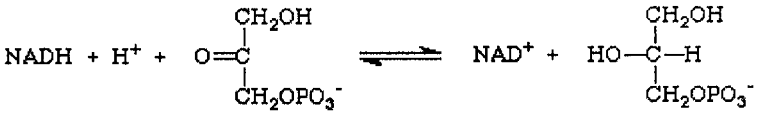
ADP and ATP are obviously among the most important substances to transport into and out of the mitochondria. Adenine nucleotide translocase exchanges matrix ATP for cytosolic ADP in their magnesium-free forms. Note that in this exchange ATP^{4-} leaves the matrix as ADP^{3-} goes in, resulting in a net loss of (1-) for the matrix. This increases the charge gradient across the membrane, and thus must be driven by the mitochondrial proton gradient. Of course P_i must also be transported across the membrane with ADP to make ATP in the matrix. This is accomplished using another transporter which co-transports a dihydrogen phosphate and a single proton in an electroneutral process. Note the addition of these two processes is equivalent to moving one proton from the cytosol to the matrix, costing the gradient one proton (moving a negative charge out is equivalent to moving a positive charge out).

The net cost of providing an ATP to the cytosol is thus four protons: three to convert ADP + P_i to ATP and one to transport ATP out of, while bringing ADP and P_i into, the matrix. This accounts for the theoretical yield of ATP: (10 H^+/NADH)/(4 H^+/ATP) = 2.5 ATP/NADH.

(This means that bacteria may get more ATP (up to 38 ATP's instead of the 32 expected in mammals).

Reducing Equivalent Shuttles: In aerobic metabolism NADH from glycolysis must be regenerated to NAD$^+$ in the mitochondria. Two shuttles are important in different tissues/organisms for this process:

- **Glycerol Phosphate Shuttle:** This shuttle is found in insect flight muscles, which sustain very high rates of aerobic glycolysis, and fast twitch muscle in humans. The NAD$^+$ is actually regenerated in the cytosol by the Glycerol-3-P DH reaction, which reduces dihydroxyacetone-P to glycerol-3-P:



$$\text{NADH} + \text{H}^+ + \text{O}{=}\underset{\displaystyle \text{CH}_2\text{OPO}_3^-}{\overset{\displaystyle \text{CH}_2\text{OH}}{\text{C}}} \rightleftharpoons \text{NAD}^+ + \text{HO}{-}\underset{\displaystyle \text{CH}_2\text{OPO}_3^-}{\overset{\displaystyle \text{CH}_2\text{OH}}{\text{C}}}{-}\text{H}$$

  The glycerol-3-P is then reoxidized to dihydroxyacetone-P by Flavoprotein dehydrogenase. This enzyme is situated on the inner mitochondrial membranes outer surface. It uses FAD to oxidize the glycerol-3-P to dihydroxyacetone-P, passing the electrons to CoQ (note the similarity to succinate DH, except for the location on the outer instead of the inner surface of the membrane). The organism can thus get 1.5 ATP equivalents for this NADH.

- **Malate-aspartate Shuttle:** This is the common shuttle in mammalian systems. It is both more complex and more efficient than the glycerol phosphate shuttle, yielding 2.5 ATP/NADH. It can be thought of as occurring in two phases:
  - o Transport of electrons from the cytosol to the mitosol, and
  - o Regeneration of the cytosolic oxaloacetate.

  In phase 1 oxaloacetate in the cytosol oxidizes NADH to NAD$^+$ using malate DH. Malate is then transported across the inner membrane in exchange for an α-ketoglutarate by Dicarboxylate translocase. Malate is then oxidized back to oxaloacetate in the matrix by Kreb's Cycle malate DH to give NADH in the matrix. In phase 2 the oxaloacetate is transaminated to aspartate, taking α-ketoglutarate to glutamate, which are transported, and the process is balanced as in the figure.

Regulation of Mitochondrial Oxidative Phosphorylation

The ETS appears to be regulated largely by the availability of ADP and NADH. For most catabolic situations [ADP] will be the controlling factor. Note that the effects of [ADP] will integrate the regulation of TCA and Glycolysis with that of ETS.

QUIZ

Identify the letter of the choice that best completes the statement or Answers the question.

1. The glycolytic pathway (Glucose to 2 Pyruvate) is found
   A. in most living organisms.
   B. in all living organisms.
   C. primarily in animals.
   D. only in eukaryotes.

Answer : B

2. In a eukaryotic cell, the enzymes of glycolysis are located in the:
   A. plasma membrane.
   B. inner mitochondrial membrane.
   C. cytosol.
   D. mitochondrial matrix.

Answer : C

3. For each molecule of glucose converted to pyruvate in the glycolytic pathway ________ molecules of ATP are used initially (Stage I) and ____ molecules of ATP are produced (Stage II) for an overall yield of ___ molecules of ATP/glucose. The "ATP math" is:
   A. -2 + 4 = 2 B. -1 + 4 = 3
   C. -2 + 5 = 3 D. -1 + 2 = 1

Answer : A

4. Phosphofructokinase, the major flux-controlling enzyme of glycolysis is allosterically inhibited by ___ and activated by ___.
   A. AMP, P_i B. ADP, AMP
   C. Citrate, ATP D. ATP, ADP

Answer : D

5. The energetic efficiency of a Chevrolet (gasoline to motion) is about 25%. By contrast, the energetic efficiency of glycolysis (glucose to ATP) *in vivo* is:
   A. less than 10%. B. about 20%.
   C. around 50%. C. more than 75%.

Answer : C

6. Mixing pure O_2 into a yeast culture growing on grape juice will cause the yeast to multiply faster and to metabolize the sugars much more rapidly. The effect on the desired final product (wine) would be:
   A. faster production of the wine.
   B. a nearly alcohol-free "beverage".
   C. little or no effect.
   D. a higher ethanol level in the wine.

Answer : B

7. The net yield of ATP in anaerobic glycolysis (ATP/glucose) in the presence of arsenate (AsO_4^{2-}) instead of phosphate is:
   A. +2. B. +1 C. 0 D. -1

   Answer : C

8. Two enzymes within a single organism that catalyze the same chemical reaction but have catalytically distinct subunits are called:
   A. apoenzymes B. antipodes
   C. coenzymes D. isozymes

   Answer: D

9. Glucose is converted to which of the following high energy intermediates in glycolysis?
   A. phosphoenolpyruvate B. 2,3-bisphosphoglycerate
   C. glucose-1-P D. fructose-1-P

   Answer: A

10. The net production of ATP from glycolysis is:
    A. 4 B. 3 C. 2 D. 1

    Answer: C

11. The significance of phosphorylating glucose is:
    A. The plasma membrane is impermeable to glucose-6-P and, therefore, keeps the substrate within the cell.
    B. A low intracellular concentration of glucose is obtained.
    C. The reaction occurs far from equilibrium.
    D. all of the above

    Answer: D

12. Which of the following reactions commits the cell to glucose metabolism?
    A. phosphoglucoisomerase
    B. phosphofructokinase
    C. fructose bisphosphate aldolase
    D. triose phosphate isomerase

    Answer: B

13. Glucokinase shares all of the following attributes with hexokinase except:
    A. product inhibition
    B. specificity for D-glucose
    C. inducible enzyme
    D. Glucokinase shares all of the above qualities with hexokinase.

    Answer: D

14. Which of the following enzymatic reactions is an example of substrate phosphorylation?
    A. hexokinase B. glucokinase
    C. phosphofructokinase D. phosphoglycerate kinase

    Answer: D

15. Enolase catalyzes which of the following types of reactions?
    A. dehydration B. hydrolytic
    C. reduction D. phosphorylation

Answer: A

16. The reaction catalyzed by which of the following enzymes has a large negative free energy?
    A. enolase B. phosphoglycerate mutase
    C. pyruvate kinase D. aldolase

Answer: C

17. Glucokinase is metabolically significant when:
    A. Glucose levels are low. B. Glucose levels are high.
    C. Glucose-6-P levels are low. D. both a and c

Answer: B

18. Which of the following molecules is a by-product of pyruvate metabolism under anaerobic conditions?
    A. acetyl CoA B. phosphoenolpyruvate
    C. 2-phosphoglycerate D. lactate

Answer: D

19. Pyruvate kinase is inhibited by which of the following molecules?
    A. AMP B. fructose-1,6-bisphosphate
    C. ATP D. ADP

Answer: C

20. Dihydroxyacetone phosphate is produced in which of the following enzymatic reactions?
    A. triose phosphate isomerase B. fructose-1-phosphate kinase
    C. triose kinase D. all of the above

Answer: C

21. FADH2 is produced in the glycolytic pathway at the step catalyzed by which of the following enzymes?
    A. glyceraldehyde-3-phosphate dehydrogenase
    B. lactate dehydrogenase
    C. succinate dehydrogenase
    D. none of the above

Answer: D

22. Formation of lactate from pyruvate involves:
    A. the oxidation of NADH to NAD B. the reduction of NAD to NADH
    C. the oxidation of FADH2 to FAD D. the reduction of FAD to FADH2

Answer: A

23. In a eukaryotic cell, most of the enzymes of the citric acid cycle are located in the
    A. mitochondrial matrix.

B. inner mitochondrial membrane.
C. intermembrane space.
D. outer mitochondrial membrane.

Answer: A

24. Pyruvate, the end product of glycolysis, enters the citric acid cycle after it has been converted to

A. acetaldehyde.
B. lactic acid.
C. acetic acid.
D. acetyl-CoA.

Answer: D

25. Most of the ATP made during cellular respiration is generated by: substrate-level phosphorylation.

A. oxidative phosphorylation.
B. glycolysis.
C. photophosphorylation.
D. The third and fourth choices are both correct.

Answer: A

26. The $FADH_2$ and NADH produced by the oxidation of one acetyl-CoA result in the synthesis of about ___ ATPs.

A. 4
B. 7
C. 11
D. 15

Answer: C

27. Energy that is released from glucose during respiration but not transferred to ATP bonds can be detected as:

A. H_2O.
B. CO_2.
C. ADP.
D. heat.

Answer: D

28. Which metabolic pathway or process is common to both aerobic and anaerobic oxidation of sugar?

A. Kreb's cycle
B. Chemiosmosis in mitochondrion
C. glycolysis
D. oxidation of NAD^+ by the electron transport chain

Answer: C

29. In contrast to resting cells, muscle tissue in a highly active metabolic state will have ______ ATP/ADP and ______ $NADH/NAD^+$.

A. high; high
B. high; low
C. low; high
D. low; low

Answer: A

30. Which of the following reactions is not a control point in the citric acid cycle?
A. Citrate synthase
B. Isocitrate dehydrogenase
C. a-Ketoglutarate dehydrogenase
D. Malate dehydrogenase

Answer: D

31. Pyruvic acid can be converted, in one enzyme-catalyzed step, to all of the following compounds except:
A. Acetyl-CoA
B. Serine
C. Lactate
D. Oxaloacetate

Answer: B

32. The following reactions in the citric acid cycle are all reversible ($DG^{o'} = 0 \pm 5$ kJ/mol) except:
A. Aconitase
B. Citrate synthase
C. Fumarase
D. Succinyl-CoA synthetase

Answer: B

33. The only phosphorylated metabolic intermediate in the citric acid cycle is:
A. *cis*-Aconitate
B. PEP
C. Phosphohistidine
D. None

Answer: D

34. Reducing equivalents produced by catabolism in the mitochondrion are transported to the cytosol for biosynthesis
A. as $FADH_2$
B. as NADH
C. as NADPH
D. indirectly as malate

Answer: D

35. Malonate (-OOC-CH2-COO-) can depress operation of the tricarboxylic acid cycle by:

A. competitively inhibiting succinate dehydrogenase
B. complexing with an essential metal ion factor
C. complexing with acetyl-CoA
D. favoring fatty acid biosynthesis

Answer: A

36. Which of the following types of chemical reactions does not occur in the TCA cycle?
A. hydrolysis
B. decarboxylation
C. phosphorylation
D. dephosphorylation

Answer: D

37. Which of the following molecules is capable of inhibiting pyruvate dehydrogenase, citrate synthase, isocitrate dehydrogenase, and a-ketoglutarate dehydrogenase?
A. succinyl CoA
B. acetyl CoA
C. ATP
D. NADH

Answer: D

38. One turn of the citric acid cycle results in how many ATP/ GTP?
A. 1
B. 3
C. 4
D. 12

Answer: D

39. Starting with one glucose molecule in glycolysis and proceeding though the citric acid cycle, how many molecules of carbon dioxide are produced?
A. 2 B. 3 C. 4 D. 6

Answer: D

40. What do the enzymes citrate synthase, isocitrate dehydrogenase, and ketoglutarate dehydrogenase have in common?
A. All are enzymes that release carbon dioxide.
B. All are enzymes that utilize NAD+.
C. All are enzymes that catalyze reduction reactions.
D. All are enzymes with large negative free energies.

Answer: D

41. Which of the following enzymes demonstrate substrate level phosphorylation?
A. isocitrate dehydrogenase
B. ketoglutarate dehydrogenase

C. succinyl CoA synthetase
D. succinate dehydrogenase

Answer: C

42. Which of the following events does not occur in the citric acid cycle?
A. A tertiary alcohol is isomerized to a secondary alcohol.
B. a trans-addition of -H and -OH across a double bond
C. a carbon-carbon condensation between a ketone and an ester
D. a-cleavage of a a-hydroxy ketone

Answer: D

43. While the TCA cycle is focused on energy production, the glyoxylate cycle is focused primarily on:
A. conserving carbon units and other intermediates for carbohydrate synthesis
B. utilizing oxidation reactions
C. carbon dioxide production
D. NADH production

Answer: A

44. The reactions of the glyoxylate cycle accomplish which of the following?
A. net synthesis of a 4-carbon dicarboxylic acid from acetyl-CoA
B. net synthesis of long chain fatty acids from TCA cycle intermediates
C. formation of phosphoenol pyruvate from oxaloacetate and ATP
D. complete oxidation of acetyl-CoA to CO2 plus reduced coenzymes

Answer: A

45. Which of the following substances is dehydrogenated to an a-keto acid in the tricarboxylic acid cycle?
A. citrate
B. glyceraldehyde-3-phosphate
C. isocitrate
D. pyruvate

Answer: C

46. Which of the following is a coenzyme involved in the conversion of succinate to fumarate in the tricarboxylic acid cycle?
A. acetyl-CoA
B. FAD
C. NAD
D. lipoic acid

Answer: B

47. The primary purpose of the glyoxylate cycle is to:
A. enable mammals, but not plants and microorganisms, to utilize fatty acids or acetate in the form of acetyl-CoA as a sole carbon source for the biosynthesis of carbohydrate

B. enable plants but not mammals to utilize fatty acids or acetate in the form of acetyl-CoA as a sole carbon source for the biosynthesis of carbohydrate
C. enable invertebrates, such as insects, to generate reducing power in the form of NADPH in the extramitochondrial cytoplasm
D. enable the transfer of reducing equivalents from cytoplasmic NADH to the mitochondrial electron transport chain in plants

Answer: B

48. The function of the enzyme dihydrolipoyl dehydrogenase (E3) in the pyruvate dehydrogenase complex is to:
A. form a hydroxyethyl derivative of the thiazole ring of enzyme-bound thiamine pyrophosphate
B. transfer an acetyl group from a bound lipoyl group on the enzyme to the thiol of CoA
C. regenerate the oxidized lipoic acid cofactor bound to one of the other enzymes in the complex
D. decarboxylate the hydroxyethyl group bound to the thiamine pyrophosphate coenzyme

Answer: C

49. Coenzymes involved in the reactions of the pyruvate dehydrogenase complex include all of the following except:
A. coenzyme A
B. thiamin pyrophosphate
C. NAD
D. biotin

Answer: D

50. The first reaction of the tricarboxylic acid cycle, the citrate synthesis reaction, involves acetyl-CoA reacting with ______________.
A. citrate
B. oxaloacetate
C. isocitrate
D. malate

Answer: B

51. Citrate synthase is a highly regulated enzyme. What is one of the ways this citrate synthase reaction is driven forward?
A. high concentration of NADH
B. low concentration of citrate
C. high concentration of succinyl-CoA
D. none

Answer: D

52. The succinyl-CoA synthase reaction used succinyl-CoA, a high-energy intermediate, to produce succinate. This reaction also directly produces _______________ in mammals.
    A. GTP
    B. ATP
    C. NADH
    D. FADH2

Answer: A

53. How many carbon atoms of an acetyl-CoA molecule are lost as CO_2 during the first reaction of the TCA cycle?
    A. 3
    B. 2
    C. 1
    D. none

Answer: D

54. The sites of regulation in the citric acid cycle involve reactions with large, negative D^0G values. These sites of regulation include:
    A. citrate synthase and aconitase
    B. isocitrate dehydrogenase and citrate synthase
    C. fumarase and isocitrate dehydrogenase
    D. a-ketoglutarate dehydrogenase and aconitase

Answer: B

55. Pyruvate dehydrogenase, which converts pyruvate to acetyl-CoA, is a tightly regulated enzyme because animals cannot:
    A. synthesize glucose from acetyl-CoA
    B. synthesize glucose from pyruvate
    C. convert acetyl-CoA to fatty acids
    D. none of the above

Answer: A

56. Pyruvate dehydrogenase is allosterically inhibited by high levels of:
    A. acetyl-CoA
    B. NADH
    C. ADP
    D. A and B

Answer: D

57. Plants and bacteria use the glyoxylate cycle to convert two acetyl-CoA molecules into:
    A. pyruvate
    B. oxaloacetate
    C. glyoxylate
    D. isocitrate

Answer: B

58. The glyoxylate cycle differs from the citric acid cycle in that the glyoxylate cycle:
    A. lacks CO_2 releasing reactions
    B. has only 5 steps
    C. consumes one molecule of acetyl-CoA per cycle
    D. A and B

Answer: D

59. A biological redox reaction always involves:
    A. a loss of electrons.
    B. a gain of electrons.
    C. a reducing agent.
    D. All of the above.

Answer: D

60. Which of the following is not a significant biological oxidizing agent?
    A. Fe^{3+}
    B. NAD^+
    C. FAD
    D. Ubiquinone (A.k.A. CoQ)

Answer: A

61. According to the Nernst equation,
    A. a negative redox potential indicates a spontaneous reaction.
    B. a positive redox potential indicates a spontaneous reaction.
    C. there is no relation between redox potential and DG.
    D. only half-reactions can actually be measured.

Answer: B

62. In a eukaryotic cell, most of the enzymes of the electron transport chain are located in the
    A. cytosol
    B. outer mitochondrial membrane.
    C. intermembrane space..
    D. inner mitochondrial membrane.

Answer: D

63. During electron transport, protons are pumped out of the mitochondrion at each of the major sites except for:
    A. *Complex I.*
    B. *Complex II.*
    C. *Complex III.*
    D. *Complex IV.*

Answer: B

64. Coenzyme Q is involved in electron transport
    - A. as a lipid-soluble electron carrier.
    - B. as a water-soluble electron donor.
    - C. as a covalently attached cytochrome cofactor.
    - D. as a water-soluble electron acceptor.

Answer: A

65. The cytochrome c oxidase complex
    - A. accepts electrons from cyt C.
    - B. donates four electrons to O_2.
    - C. produces 2 H_2O per O_2 reduced.
    - D. All of the above are correct.

Answer: D

66. Uncoupling agents of oxidative phosphorylation:
    - A. allow electron transport to continue but prevent the phosphorylation of ADP to ATP
    - B. prevent electron transport from occurring but allow the phosphorylation of ADP to ATP
    - C. block both electron transport and the phosphorylation of ADP to ATP
    - D. are agents like rotenone and cyanide that block electron transport at specific carriers

Answer: A

67. If the insecticide rotenone is added to a system capable of carrying out electron transport and oxidative phosphorylation, the step which is inhibited involves:
    - A. the NADH-UQ reductase
    - B. cytochrome oxidase
    - C. coenzyme Q-cytochrome c reductase
    - D. succinate-coenzyme Q reductase

Answer: A

68. Ethanol + NAD+ --> acetaldehyde+ NADH + H+

    A B C D

    Which of the above molecules are being oxidized and which are reduced in the reaction at equilibrium?
    - A. oxidized A, B; reduced C, D
    - B. oxidized A, C; reduced B,D
    - C. oxidized A, D; reduced B, C
    - D. oxidized B, C; reduced A,C

Answer: C

69. Ethanol + NAD+ --> acetaldehyde + NADH + H+
How many electrons are being transferred in the above redox reaction?
A. 0 B. 1 C. 2 D. 3

Answer: C

70. The 2 e- carrier coenzyme Q is able to accept electrons for the 1 e- donor Fe in Complex I because:
A. coenzyme Q can accept electrons one at a time because it forms a stable semiquinone
B. reduced Fe atoms can donate their electrons at exactly the same time
C. coenzyme Q contains two Fe atoms, so can be reduced at either of them separately
D. CoQ does not accept electrons from an Fe-S complex, but from FMNH2

Answer: A

71. Chemiosmotic coupling describes:
A. how the energy from the electron transport chain is used to generate an electrochemical gradient for protons, which is used to drive ATP synthesis
B. how the electrons are passed through the electron transport chain by coenzymes, iron-sulfur centers, and cytochromes
C. how the oxidation of NADH and FADH2 results in utilization of oxygen
D. how ATP synthase rotates to generate ATP

Answer: A

72. How does cytochrome c shuttle electrons between Complexes III and IV?
A. Its heme prosthetic group swings on a long arm between the two proteins
B. It donates the electrons to oxygen, which then goes to Complex IV to be reduceD.
C. It only loosely associates with the inner membrane and floats in the intermembrane space between the two protein complexes.
D. It is a coenzyme with a long hydrophobic tail that diffuses laterally through the inner membrane between the two protein complexes.

Answer: C

73. Which complex along the electron transport chain does not contribute to the proton gradient?
A. Complex I B. Complex II C. Complex III D. Complex IV

Answer: B

74. Which of the complexes of the electron transport chain shares its enzymatic activity with the citric acid cycle?
A. Complex I B. Complex II C. Complex III D. Complex IV

Answer: B

75. Where do the electrons from the NADH and succinate end up after electron transport in the mitochondria?
    - A. They reduce ADP to ATP.
    - B. They reduce the Fe3+ to Fe2+ in the heme group of hemoglobin.
    - C. They are pumped across the inner mitochondrial membrane.
    - D. They reduce oxygen to water.

Answer: D

76. Uncoupling agents are compounds that block ATP synthesis while allowing the respiration of the electron chain to continue. Which of the following would explain this?
    - A. They are compounds that move protons back across the inner mitochondrial membrane in the same direction as the proton gradient.
    - B. They bind to Complex II and stop the flow of protons through ATP synthase
    - C. They inactivate cytochrome c
    - D. They oxidize NADH and succinate

Answer: A

77. Which of the following compounds serves as a positive allosteric activator of glycolysis?
    - A. glucose-6-phosphate
    - B. pyruvate
    - C. dihydroxy acetone phosphate
    - D. fructose-1,6-bisphosphate

Answer: D

78. In the pentose phosphate pathway, from glucose-6-phosphate to ribulose-5-phosphate, which of the following coenzymes, intermediates, and enzymes are involved?
    - A. glucose-6-phosphate dehydrogenase, NADH, gluconolactone hydrolase, 6-phosphogluconate dehydrogenase, ribulose-5-phosphate epimerase
    - B. 6-phosphogluconate dehydrogenase, gluconolactone hydrolase, NADPH, glucose-6-phosphate dehydrogenase
    - C. NADPH, 6-phosphogluconate dehydrogenase, transketolase, glucose-6-phosphate dehydrogenase, gluconolactone hydrolase
    - D. NADPH, glucose-6-phosphate dehydrogenase, gluconolactone hydrolase, ribulose-5-phosphate epimerase, transaldolase

Answer: B

79. Amylopectin, when hydrolyzed in the presence of a-amylase, yields maltose and limit dextrins. However, amylose, under the same conditions,

almost quantitatively produces maltose. This difference in behavior is best explained by:

A. the greater solubility of amylose in water
B. differences in the molecular weights of amylose and amylopectin
C. the presence of more glucose units in the amylose molecule
D. the presence of substantial numbers of a-1-6 linkages in amylopectin and none in amylose

Answer: D

80. In the pentose phosphate pathway, NADPH is produced at the step catalyzed by:

A. phosphopentose isomerase
B. gluconolactonase
C. 6-phosphogluconate dehydrogenase
D. transketolase

Answer: C

81. Which of the following is NOT a major storage molecule for animal tissues?

A. protein
B. glycogen
C. triaclyglycerols
D. cellulose

Answer: D

82. Which of the following is NOT true regarding the pentose phosphate pathway?

A. It is an alternative process to glycolysis for oxidation of glucose
B. It functions to provide NADPH for reductive biosynthesis.
C. It functions to provide ribose-5-phosphate for nucleotide and nucleic acid biosynthesis.
D. It generates NADH for utilization by the electron transport chain.

Answer: D

83. Glycogen catabolism is initiated by the action of:

A. starch phosphatase
B. hexokinase and maltase
C. a-amylase and a-(1, 6)-glucosidase (debranching enzyme)
D. phosphofructokinase

Answer: C

84. One of the factors that drives the formation of UDP-glucose from glucose-1-phosphate and UTP is that:

A. UTP has a much higher free energy of hydrolysis than ATP.

B. The presence of a phosphate group on the 1-position of glucose-1-phosphate causes a steric strain which is relieved when UDP-glucose is formeD.

C. UDP-glucose has a much higher free energy of hydrolysis than either UTP or glucose-1-phosphate

D. One of the products of the reaction is inorganic pyrophosphate which is subsequently hydrolyzed to inorganic phosphate

Answer: D

85. Which of the following is **not** a mechanism for altering the flux of metabolites through the rate-determining step of a pathway?

A. Covalent modification of the enzyme.

B. Genetic control of the enzyme concentration.

C. Diffusional coupling between adjacent active sites.

D. Allosteric control of the enzyme activity.

Answer: C

86. The breakdown of glycogen to form glucose occurs

A. in the liver by phosphorolysis.

B. in the muscles by phosphorolysis.

C. in the liver by hydrolysis.

D. A and B are correct

Answer: D

87. The precursor to glycogen in the glycogen synthase reaction is

A. glucose-1-P.

B. glucose-6-P.

C. UDP-glucose.

D. UTP-glucose.

Answer: C

88. The characteristic enzymes of gluconeogensis are found in the cytosol, except for

A. pyruvate carboxlyase, which is in the mitrochondria.

B. fructose-1,6-bisphosphatase, which is in the mitochondria.

C. glucose-6-phosphatase, which is in the mitrochondria.

D. fructose-1,6-bisphosphatase, which is in the glycogen granule.

Answer: A

89. Fructose-2,6-bisphosphate (F2,6P)

A. inhibits phosphofructokinase.

B. activates fructose-1,6-bisphosphatase.

C. activates phosphofructokinase.

D. None

Answer: C

90. The regulation of the glycolytic pathway involves
    A. feedback inhibition by ATP.
    B. allosteric inhibition by ATP.
    C. allosteric stimulation by ADP.
    D. All are correct.

Answer: D

91. During gluconeogenesis in the liver, pyruvate is converted to PEP
    A. in two enzyme-catalyzed steps.
    B. using two equivalents of ATP.
    C. directly by the reversibility of pyruvate kinase.
    D. A and B are correct

Answer: D

92. Glycolysis takes place in:
    A. mitochondria
    B. vacuole
    C. chloroplasts
    D. cytoplasm

Answer: D

93. If one molecule of glucose is completely oxidised to H_2O and CO_2, Considering 3ATP equivalent/ NAD a total of:
    A. 38 molecules of ATP may be produced
    B. 32 molecules of ATP may be produced
    C. 34 molecules of ATP may be produced
    D. 36 molecules of ATP may be produced

Answer: A

94. If one molecule of glucose is completely oxidised to H_2O and CO_2, Considering 2.5ATP equivalent/ NAD a total of:
    A. 38 molecules of ATP may be produced
    B. 32 molecules of ATP may be produced
    C. 34 molecules of ATP may be produced
    D. 36 molecules of ATP may be produced

Answer: B

95. The Krebs cycle and oxidative phosphorylation take place in:
    A. vacuole
    B. mitochondria
    C. cytoplasm
    D. chloroplasts

Answer: B

96. The net number of ATP molecules produced when one molecule of glucose passes through the anaerobic stage of respiration is:

A. 1 B. 2 C. 3 D. 4

Answer: B

97. When one molecule of high energy NAD enters the electron transfer chain during oxidative phosphorylation, the number of ATP molecules formed is:

A. 1 B. 0 C. 3 D. 4

Answer: C

98. The stage of respiration during which carbon dioxide is evolved is:

A. the Krebs cycle
B. phosphorylation
C. the electron transport chain
D. oxidative phosphorylation

Answer: A

99. The stage of respiration during which water is evolved is:

A. the Krebs cycle
B. oxidative phosphorylation
C. phosphorylation
D. the electron transport chain

Answer: B

100. Energy to convert glucose to hexose bisphosphate in phosphorylation is provided by:

A. NAD B. AMP C. ADP D. ATP

Answer: D

101. The stage of respiration in which glucose is converted to pyruvate is:

A. the Krebs cycle B. phosphorylation
C. oxidative phosphorylation D. the electron transport chain

Answer: B

102. During anaerobic respiration in yeast, glucose is converted to:

A. ethanol and carbon dioxide
B. oxygen
C. oxygen and water
D. water and carbon dioxide

Answer: A

103. Which of the following occurs in both photosynthesis and respiration?

A. chemiosmosis B. glycolysis
C. calvin cycle D. krebs cycle

Answer: A

104. Which of the following statements is FALSE?

A. glycolysis can occur with or without oxygen
B. glycolysis occurs in the mitochondria

C. glycolysis is the first step in both aerobic and anaerobic respiration
D. glycolysis produces 2 ATP, 2 NADH, and 2 pyruvate

Answer: B

105. This process uses NADH and $FADH_2$ to produce ATP
A. oxidative phosphorylation
B. fermentation
C. glycolysis
D. krebs cycle

Answer: A

106. This process begins with the production of Acetyl-CoA:
A. chemiosmosis
B. glycolysis
C. fermentation
D. krebs cycle

Answer: D

107. Cramps during exercise are caused by:
A. alcohol fermentation
B. glycolysis inhibition
C. lactic acid fermentation
D. chemiosmosis

Answer: C

108. Oxidative phosphorylation is also known as:
A. chemiosmosis
B. glycolysis
C. fermentation
D. electron transport chain

Answer: D

109. The final electron acceptor during oxidative phosphorylation is:
A. oxygen
B. water
C. carbon dioxide
D. ATP

Answer: B

110. Which of the following processes produces the most ATP?
A. glycolysis
B. oxidative phosphorylation
C. fermentation
D. krebs cycle

Answer: B

111. Which of the following is necessary for oxidative phosporylation to occur?

A. ATP
B. oxygen
C. carbon dioxide
D. lactic acid

Answer: B

112. Which of the following is the products of the Krebs cycle?
A. ATP
B. NADH
C. FADH
D. All

Answer: D

113. Lactic acid fermentation occurs in
A. bread dough.
B. any environment containing oxygen.
C. muscle cells.
D. mitochondria.

Answer: C

114. The conversion of pyruvic acid into lactic acid requires
A. alcohol.
B. oxygen.
C. ATP.
D. NADH.

Answer: D

115. The energy of the electrons passing along the electron transport chain is used to make
A. lactic acid.
B. citric acid.
C. alcohol.
D. ATP.

Answer: D

116. Breathing heavily after running a race is your body's way of
A. making more citric acid.
B. repaying an oxygen debt.
C. restarting glycolysis.
D. recharging the electron transport chain.

Answer: B

117. The energy needed to win a 2-minute footrace is produced mostly by
A. lactic acid fermentation.
B. cellular respiration.
C. using up stores of ATP.
D. breaking down fats.

Answer: A

118. Which statement mainly explains why even well-conditioned athletes have to pace themselves for athletic events that last several hours?
    - A. Lactic acid fermentation can cause muscle soreness.
    - B. Heavy breathing is needed to get rid of lactic acid.
    - C. Cellular respiration releases energy more slowly than fermentation does.
    - D. all of the above

Answer: C

119. All of the following are sources of energy during exercise EXCEPT
    - A. stored ATP.
    - B. alcoholic fermentation.
    - C. lactic acid fermentation.
    - D. cellular respiration.

Answer: B

120. The products of photosynthesis are the
    - A. products of cellular respiration.
    - B. reactants of cellular respiration.
    - C. products of glycolysis.
    - D. reactants of fermentation.

Answer: B

121. Which of the following is NOT a stage of cellular respiration?
    - A. fermentation
    - B. electron transport
    - C. glycolysis
    - D. Krebs cycle

Answer: A

122. Which of the following is the correct sequence of events in cellular respiration?
    - A. glycolysis - fermentation - Krebs cycle
    - B. Krebs cycle - electron transport - glycolysis
    - C. glycolysis -Krebs cycle - electron transport
    - D. Krebs cycle - glycolysis - electron transport

Answer: C

123. The electron transport chain can be found in

A. prokaryotes. B. animals.
C. plants. D. all

Answer: D

124. Which of the following passes high-energy electrons into the electron transport chain?

A. NADH and $FADH_2$ B. ATP and ADP
C. citric acid D. acetyl - CoA

Answer: A

Complete each sentence or statement.

1. The activation of a later step in a reaction sequence by the product of an earlier step is called _______.

   Answer: feed-forward activation

2. An enzyme that transfers a phosphoryl group between ATP and another molecule is ________.

   Answer: kinase

3. ________ is the synthesis of glucose from noncarbohydrate precursors.

   Answer: Gluconeogenesis

4. A graphical convention for specifying molecular configuration in which horizontal lines represent bonds that extend above the plane of the paper and vertical bonds extend below the plane of the paper is a/an ________.

   Answer: Fischer projection

5. ________ is a term for any anaerobic catabolic process.

   Answer: Fermentation

6. ________ is the slowest step in a multi-step sequence, such as a metabolic pathway, whose rate determines the rate of the entire sequence.

   Answer: Rate-determining reaction or Rate-limiting step

7. A pathway for glucose degradation that yields ribose-5-phosphate and NADPH is the ________.

   Answer: pentose phosphate pathway

8. The enzymatic degradation of glycogen to glucose-1-phosphate is called ________.

   Answer: glycogenolysis

9. A/An ________ is a drawing of a sugar ring in which ring bonds that project in front of the plane of the paper are represented by heavy lines and ring bonds that project behind the plane of the paper are represented by light lines.

   Answer: Haworth projection

10. A ketose is a sugar with the structure of a/an ________.

    Answer: ketone

11. A/An ________ is an extracellular complex of protein and glycosaminoglycans.

    Answer: proteoglycan

12. ________ is the rate of flow of metabolites through a metabolic pathway.

    Answer: Flux

13. An unbranched polysaccharide consisting of alternating residues of an amino sugar and a sugar acid is a/an ________.

    Answer: glycosaminoglycan

14. ________ is a reaction whose D^{0}G value is close to zero, so that it can operate in either direction depending on the substrate and product concentrations.

Answer: Near-equilibrium reaction

15. The ________ is the greatly increased sugar consumption of yeast grown under anaerobic conditions compared to that of yeast grown under aerobic conditions.

Answer: Pasteur effect

16. A reaction that replenishes the intermediates of a metabolic pathway is a/an ________.

Answer:anaplerotic reaction

17. The gel-like solution of enzymes, substrates, cofactors, and ions in the interior of the mitochondrion is called ________.

Answer: mitochondrial matrix

18. ________ is a membrane-bounded plant organelle in which the reactions of the glyoxylate cycle take place.

Answer: Glyoxisome

19. A/An ________ is a group of noncovalently associated enzymes that catalyze two or more sequential steps in a metabolic pathway.

Answer: multienzyme complex

20. A variation of the citric acid cycle in plants that allows acetyl-CoA to be converted quantitatively to gluconeogenic precursors is the ________.

Answer: glyoxylate cycle

21. The pyruvate dehydrogenase complex decarboxylates pyruvate and attaches the remaining two-carbon group to ________.

Answer: coenzyme A or CoA

22. The enzyme that catalyzes the condensation of acetyl-CoA with oxaloacetate is ________.

Answer: citrate synthase

23. The two acetyl carbons that enter each round of the citric acid cycle are eventually released as two molecules of ________.

Answer: carbon dioxide

24. Pyruvate is converted to ________ by the action of pyruvate carboxylase.

Answer: oxaloacetate

25. Reactions catalyzed by ________ convert some amino acids to intermediates of the citric acid cycle.

Answer: transaminases

26. NAD+ is reduced to ________ in three of the reactions of the citric acid cycle.

Answer: NADH

27. A series of hydrogen-bonded water molecules and protein groups that can relay protons from one site to another is a/an ________.

Answer: proton wire

28. An expression of the relationship between the actual and standard reduction potential of a substance is the ________.

Answer: Nernst equation

29. ________ is the free energy of the electrochemical proton gradient that forms during electron transport.

Answer: Protonmotive force

30. ________ is the generation of heat by muscular contraction or by metabolic reactions.

Answer: Thermogenesis

31. The invaginations of the inner mitochondrial membrane are ________.

Answer: cristae

32. A/An ________ is a chemical reaction in which one substance is reduced and another substance is oxidized.

Answer: redox reaction or oxidation-reduction reaction

33. The cyclic flow of electrons involving a semiquinone intermediate in Complex III of mitochondrial electron transport is called the ________.

Answer: Q cycle

34. Respiration is the metabolic phenomenon whereby organic molecules are ________.

Answer: oxidized

35. The ________ in mitochondria is equivalent to the cytosol in ionic composition.

Answer: intermembrane space

36. ________ is the single oxidation or reduction process, involving the reduced and oxidized forms of a substance, that must be combined with another to form a complete oxidation-reduction reaction.

Answer: Half-reaction

37. The postulate that the free energy of electron transport is conserved in the formation of a transmembrane proton gradient that can be subsequently used to drive ATP synthesis is the ________.

Answer: chemiosmotic theory

38. A reducing agent, also called a/an ________, is a substance that can donate electrons, thereby becoming oxidized.

Answer: reductant

39. A/An ________ is a substance that allows the proton gradient across a membrane to dissipate without ATP synthesis so that electron transport proceeds without oxidative phosphorylation.

Answer: uncoupler or uncoupling agent

40. ________ is a technique for reconstructing three-dimensional structures by analyzing electron micrographs of consecutive tissue slices.

Answer: Electron tomography

41. ________ is a protein that carries electrons via a prosthetic Fe-containing heme group.

Answer: Cytochrome

42. ________ is the double-membrane-enveloped eukaryotic organelle in which aerobic metabolic reactions occur.

Answer: Mitochondrion

43. A group that can undergo an oxidation-reduction reaction is a/an ________.

Answer: redox center

44. ________ is a measure of the tendency of a substance to gain electrons (to be reduced) under standard conditions.

Answer: Standard reduction potential

45. A measure of the tendency of a substance to gain electrons is its ________.

Answer: reduction potential

46. ________ is an electron micrographic technique for visualizing the shapes of large particles that are embedded in ice so that they retain their native shape to a greater extent than in convention electron microscopy.

Answer: Cryoelectron microscopy

Chapter **10**

Lipid Metabolism

Pathways of the Fat System (Fig 10.1)

Lipolysis: hydrolysis of triacylglycerol to glycerol and free fatty acids

β-Oxidation: mitochondrial oxidation of fatty acids -----> Energy!

Ketogenesis: synthesis of ketone bodies.

Lipogenesis: fatty acid synthesis.

Esterification: attachment of fatty acids to glycerol to form triglycerides.

Cholesterolgenesis: synthesis of cholesterol .

Steroidogenesis: synthesis of steroid hormones from cholesterol.

Interactions of Fat Metabolism Pathways:

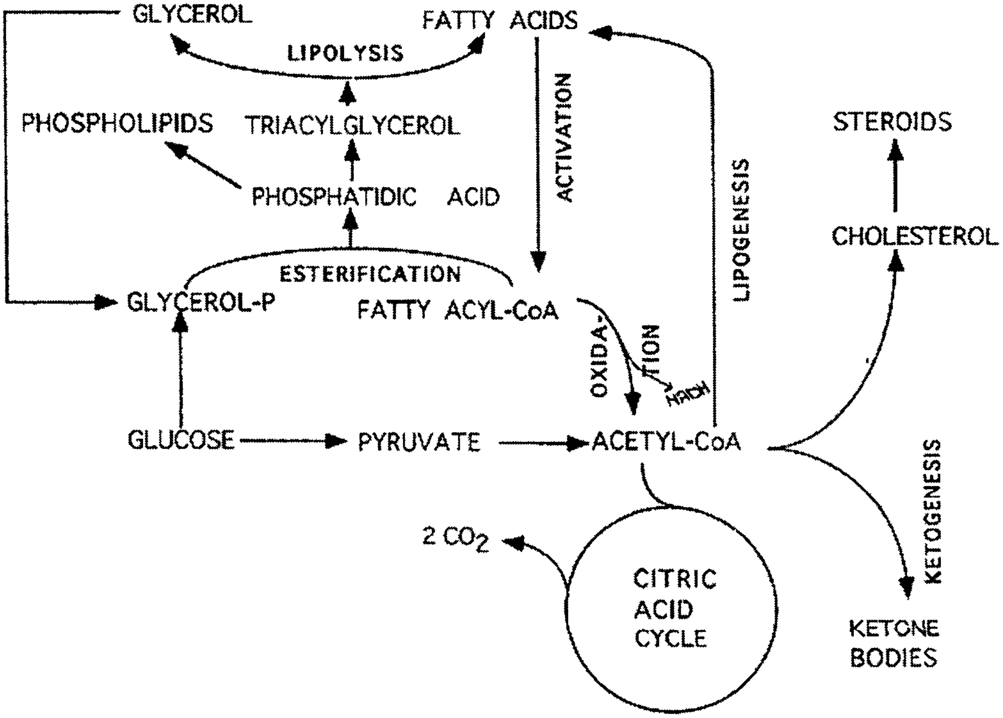

GLYCEROL
FATTY ACIDS
LIPOLYSIS
PHOSPHOLIPIDS
TRIACYLGLYCEROL
ACTIVATION
STEROIDS
PHOSPHATIDIC ACID
LIPOGENESIS
CHOLESTEROL
ESTERIFICATION
GLYCEROL-P
FATTY ACYL-CoA
OXIDA-TION
NADH
GLUCOSE
PYRUVATE
ACETYL-CoA
KETOGENESIS
2 CO2
CITRIC ACID CYCLE
KETONE BODIES

Fig. 10.1 : Overview of Lipid Metabolisms

Fat Metabolism in Specific Tissues: (Fig. 10.2)

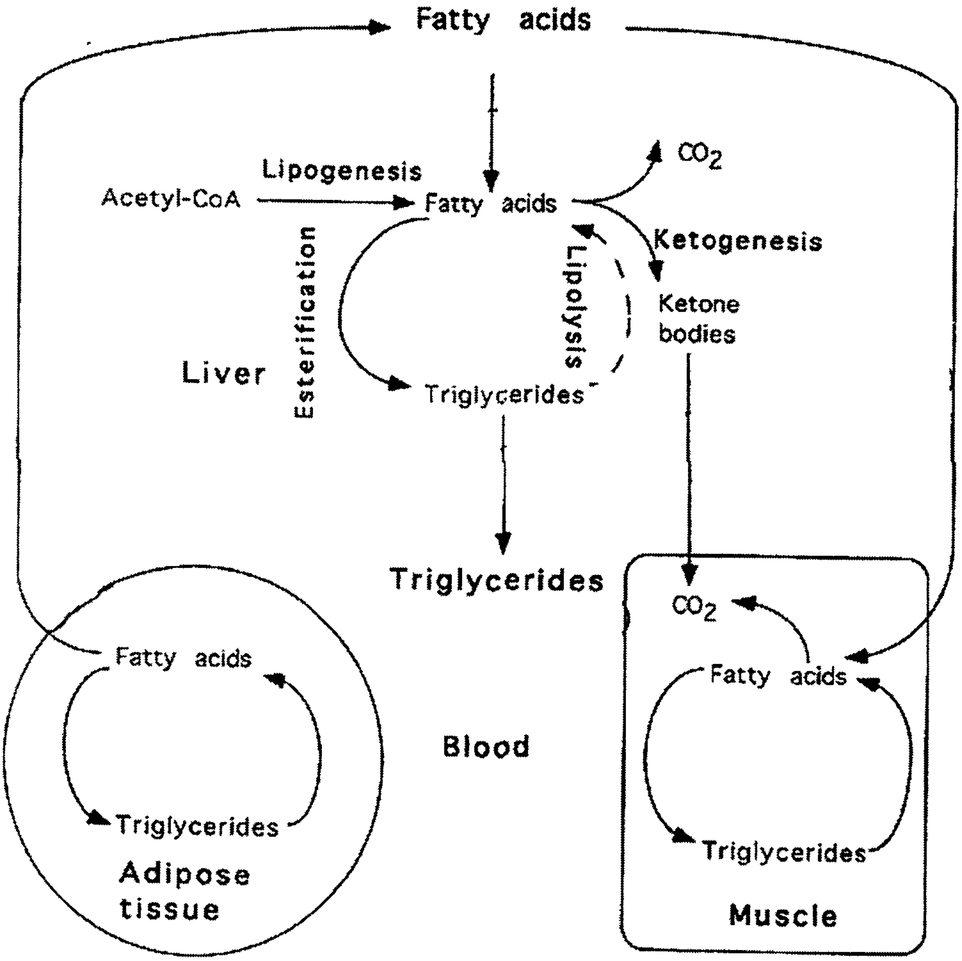
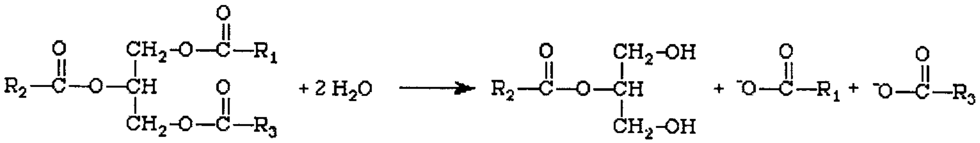

Fatty acids
Lipogenesis
Acetyl-CoA
Fatty acids
CO_2
Ketogenesis
Esterification
Lipolysis
Ketone bodies
Liver
Triglycerides
Triglycerides
CO_2
Fatty acids
Fatty acids
Blood
Triglycerides
Triglycerides
Adipose tissue
Muscle

Fig. 10.2 : Schematic view of Fat metabolism Linked to various Tissues

- **Liver**: oxidizes fats, produces ketones, exports triglycerides.
- **Muscle**: uses fats and ketones as an energy source.
- **Adipose Tissue**: stores fats as triacylglycerol and releases fatty acids as needed for energy.
- **Mature RBC's** (no mitochondria) / **Brain** (lack of enzymes): do not use fats for energy.

Lipolysis

Fats Come From Two Main Sources: stored body fat and dietary fat. Dietary fat must first be emulsified to increase its surface area for contact with the water soluble lipases. This occurs largely in the duodenum after mixing with the bile acids, a family of cholesterol derived detergents. Triacylglycerols can then be hydrolyzed by pancreatic lipase to free fatty acids and 2-monoacylglycerol:



The fatty acids and monoacylglycerol are absorbed by the intestinal cells, converted to fatty acyl CoA and reassembled into triacylglycerols. The triacyl glycerols then assemble with phospholipids and lipoproteins to form chylomicrons for transport through the lymph and blood to the tissues.

When the chylomicrons reach tissue cells the triacylglycerols are again hydrolyzed by lipoprotein lipase to fatty acids which can be taken up by the peripheral tissue cells. In adipose cells the fatty acids are then converted into fatty acyl CoA's and combined into triacylglycerols for storage. Alternatively the fatty acids can be broken down for energy using the various oxidative pathway in specific tissues.

Oxidation of Fatty Acids

Fatty acids obtained by hydrolysis of fats undergo different oxidative pathways designated as (α), beta (β) and omega (ω) pathways.

α-oxidation

α-Oxidation of fatty acids has been found in certain tissues especially in brain tissue of mammals and plant systems. It does not require CoA intermediates and no high-energy phosphates are generated. This type of oxidation results in the removal of one carbon at a time from the carboxyl end of the fatty acid. The physiological role of α-oxidation in plants is not yet fully established but it has been suggested that it may be involved in the degradation of long chain fatty acids as observed in many animal tissues. α-Oxidation is clearly the main source of the odd-carbon fatty acids and their derivatives that occur in some plant lipids. In this process, sequential removal of one carbon at a time from free fatty acids of chain length ranging from C13 to C18 occur.

ω-Oxidation

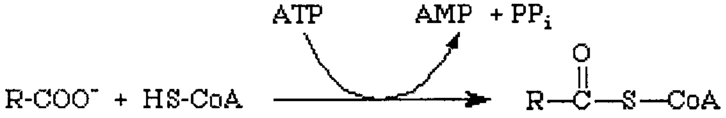
ω-Oxidation is normally a very minor pathway brought about by hydroxylase enzymes involving cytochrome P-450 in the endoplasmic reticulum. Fatty acids with oxygen function (alcoholic or carboxyl) at the methyl terminal end (ω-end) are formed by ω-oxidation and frequently occur as constituents of cutin and suberin. The requirements for the oxygenase-mediated conversion of a ω-methyl fatty acyl CoA into a ω-hydroxymethyl fatty acyl CoA are molecular oxygen, reduced pyridine nucleotide and a non-heme iron protein in higher plants.

β-Oxidation of Fatty Acids

In 1904, Franz Knoop made a critical contribution to the elucidation of the mechanism of fatty acid oxidation and demonstrated that most of the fatty acids are degraded by oxidation at the β-carbon. β-Oxidation of fatty acids takes place in mitochondria. Fatty acids are activated before they enter into mitochondria for oxidation.

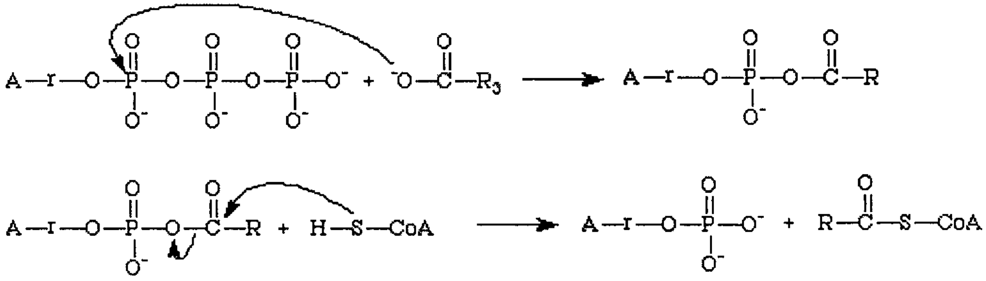
Free fatty acids are introduced into the cytosol, but β-oxidation occurs in the mitosol. Two situations occur.

- **Short to medium length fatty acids** are permeable to the mitochondrial membrane. They are activated to fatty acyl CoA derivatives in the mitochondrial matrix by **Butyryl-CoA Synthetase**:

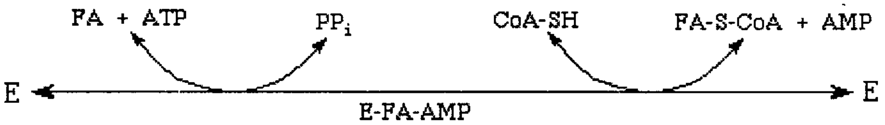


Note that two ATP equivalents are required: the phosphoanhydride and thioester bonds are of similar free energies, so a second phosphoanhydride bond is also hydrolyzed to drive the reaction to completion.

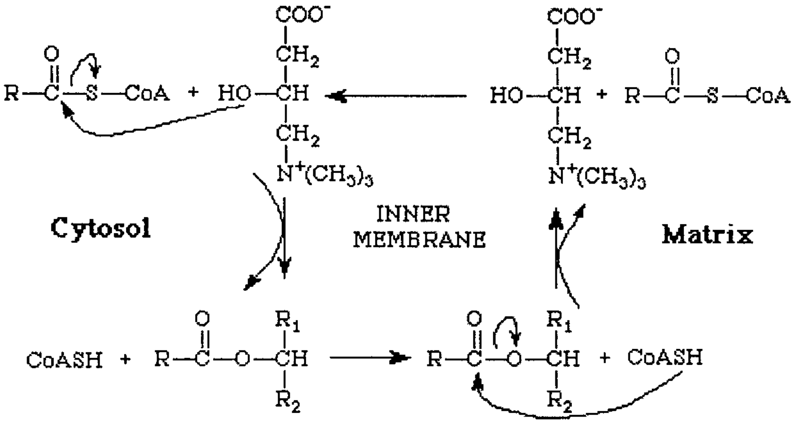
- **Long chain fatty acids** are are bound to Fatty acid binding protein for transport within the cytosol. They are impermeable to the inner mitochondrial membrane. They are thus esterified in the cytosol by microsomal **Fatty acyl CoA synthetase** in a reaction identical to the one shown above. Again the reaction is driven by the hydrolysis of pyrophosphate. The enzyme involves an acyl AMP intermediate:



with Ping Pong Bi Uni-Uni Bi kinetics:



Carnitine Carrier: The resulting acyl CoA ester is still not permeable to the mitochondrial membrane so a carrier system is needed. In this system the fatty acyl group is transferred from CoA-S to carnitine, diffuses across the membrane, and then transferred back to another CoA-S within the matrix:



The carnitine transport step across the inner membrane is the slow step and **flux generating step for β-oxidation** of long chain fatty acids. Note that this system maintains separate pools of CoASH in the cytosol vs. the matrix.

Once inside the mitochodrial matrix fatty acyl CoA can be broken down in the matrix by the fatty acid β-oxidation cycle.

β-Oxidation of Free Fatty Acids With an Even Number of Carbons (Fig. 10.3)

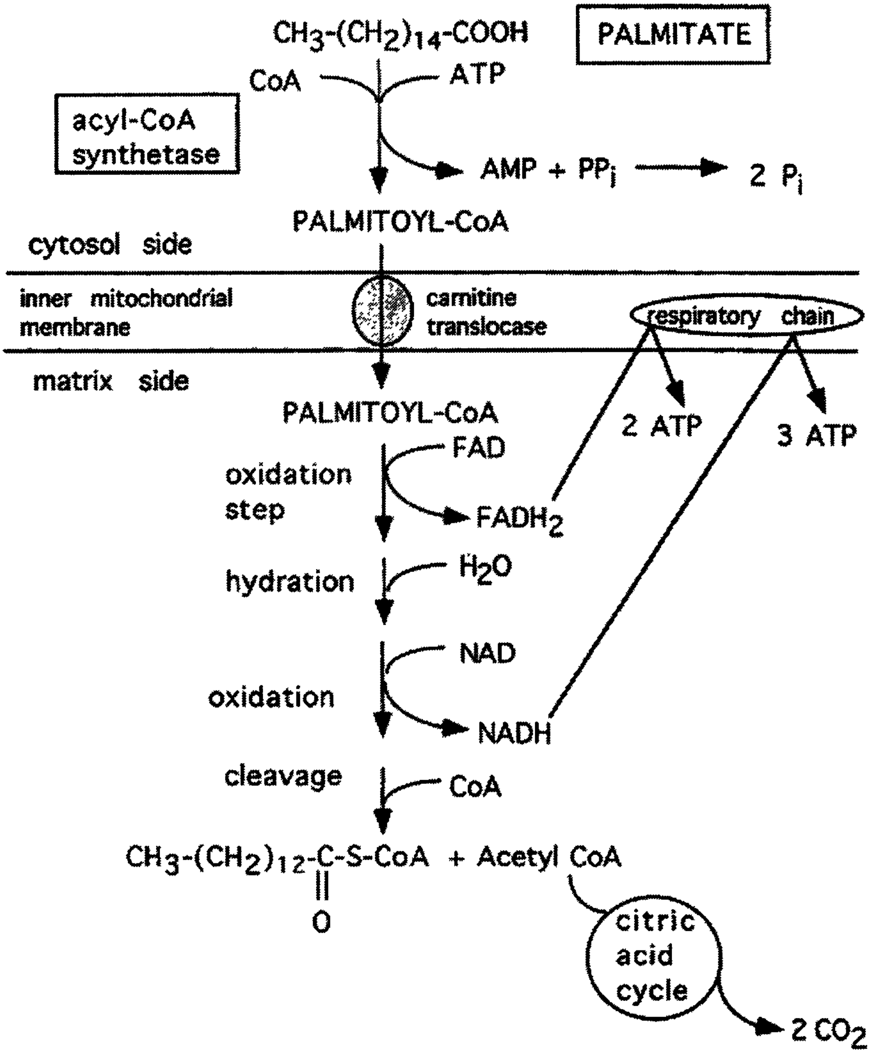

CH3-(CH2)14-COOH
PALMITATE
CoA
ATP
acyl-CoA synthetase
AMP + PPi
2 Pi
PALMITOYL-CoA
cytosol side
inner mitochondrial membrane
carnitine translocase
respiratory chain
matrix side
PALMITOYL-CoA
2 ATP
3 ATP
FAD
oxidation step
FADH2
hydration
H2O
NAD
oxidation
NADH
cleavage
CoA
CH3-(CH2)12-C-S-CoA + Acetyl CoA
O
citric acid cycle
2 CO2

Fig. 10.3 : β-oxidation of Palmitoyl - CoA

Reactions 2 through 5 recycle to remove consecutive 2-carbon units. On the final cycle, 2 acetyl CoA molecules are formed. Thus, a 16 carbon fatty acid needs to cycle only 7 times.

- The first reaction in β-oxidation of acyl CoA is the formation of trans $\Delta 2$-enoyl CoA or α, β-unsaturated acyl CoA in presence of acyl-CoA dehydrogenase and the coenzyme, FAD.
- The next step is the hydration of the double bond between C-2 and C-3 by enoyl CoA hydratase with the formation of β-hydroxy acyl CoA.
- In the third step, the β-hydroxy acyl CoA is dehydrogenated in the presence of β-hydroxy acyl CoA dehydrogenase and NAD+ forming β -ketoacyl CoA.
- In the last step of β-oxidation, β-ketoacyl CoA reacts with coenzyme A in the presence of the enzyme, thiolase. The products of this reaction are acetyl CoA and an acyl CoA containing two carbons less than the original acyl CoA molecule that underwent oxidation.

By the above steps of β-oxidation fatty acids are completely degraded to acetyl CoA units. The acetyl CoA formed from fatty acids can be oxidised to carbon dioxide and water via citric acid cycle.

Energetics of β oxidation

The energetics or the energy conserved in terms of ATP by oxidation of a molecule of palmitic acid is given below:

Palmitic acid (16 carbons) undergoes β-oxidation forming eight molecules of acetyl CoA by undergoing seven β-oxidation spirals. When one cycle of β -oxidation takes place, one molecule of FADH2, one molecule of NADH and one molecule of acetyl CoA are produced. Electrons from these reducing equivalents (FADH2 and NADH) are transported through the respiratory chain in mitochondria with simultaneous regeneration of high-energy phosphate bonds. Mitochondrial oxidation of FADH2 eventually results in the net formation of about 1.5 ATP. Likewise, oxidation of electrons from NADH yields 2.5 molecules of ATP. Hence, a total of four ATP molecules are formed per cycle and ten molecules of ATP are formed through Krebs's cycle from each molecule of acetyl CoA.

8 Acetyl CoA through TCA cycle yield (8x10) = 80 ATP
7 β-oxidation spiral reactions yield (7x4) = 28 ATP

Total 108 ATP

ATP utilized in the initial step = 2 ATP
Hence, complete oxidation of palmitic acid yields **106 ATP.**

If we look at ATP/C we get 106/16= 6.63, while for glucose we get 32/6= 5.33, and for hexanoate: 36/6= 6. Thus, as expected, the fatty acids, being more reduced on average, give more energy per carbon and per gram. Along with the fact that they are stored without water of hydration, unlike carbohydrates, we can see their advantage as energy storage molecules for mobile organisms.

Another measure of fuel use is the P/O ratio, the number of ATP's generated for each oxygen atom consumed. For palmitate P/O = 106/46 = 2.3. As a comparison the P/O for glucose = 32/12 = 2.67. Note that, *By this measure glucose is the better fuel in situation where oxygen is limiting because glucose will give more ATP's per ml of oxygen.*

β Oxidation of Odd Chain Fatty Acids

Most biological fatty acids are of even-numbered carbon chains. However, some organisms, particularly in the arctic marine environment, have a relatively high odd-chain component. Thus in organisms such as traditional Eskimo (Innuit) and polar bears eating lots of seal blubber and fish, odd-chain fatty acids can constitute a significant dietary component. These fatty acids are handled normally through β-oxidation until the last turn, where pentyl-CoA is cleaved into acetyl-CoA and propionyl-CoA. The propionyl-CoA is converted through a number of steps to succinyl-CoA. These steps involve addition of carbon dioxide (with ATP energy) and an isomerization requiring cobalamin derived from vitamin B_{12}. The succinyl-CoA can then be metabolized normally via the TCA cycle to malate, then to PEP and then to either 2-PGA for gluconeogenesis or to Pyruvate for energy production. (Propionate metabolism is also important to ruminants, since it is produced as a fermentation product by their symbiotic bacteria from plant matter.)

End products are propionyl CoA and acetyl CoA caused by cleavage of a 5-carbon fatty acid during the final cycle.

Propionyl CoA Carboxylase

Propionyl CoA + ATP + CO_2 -----> Methylmalonyl CoA + AMP + PP_i

Methylmalonyl CoA Mutase

Methylmalonyl CoA -----> Succinyl CoA (enters Citric Acid Cycle)

Energy Production

35 ATP (7 cycles β-oxidation, NADH and $FADH_2$ produced)

72 ATP (6 cycles β-oxidation, accounts for 12 carbon atoms)

Acetyl CoA + 1 Succinyl CoA 18 ATP (produced in the last step from the remaining 5 carbons, enters TCA cycle)

Total : 125 ATP

-1 ATP = **124 ATP (Net)**

β-Oxidation of unsaturated fatty acids

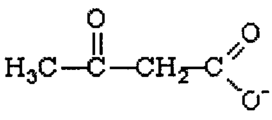
One might think that since an unsaturated fatty acid is created during *beta*-oxidation that unsaturated fatty acids should be handled easily by this system. However two problems occur due to the high level of specificity of the enzymes involved:

- First the double bond can occur in the wrong position, and
- Second, unsaturated fatty acids are commonly cis-, whereas *beta*-oxidation uses trans-fatty acids.

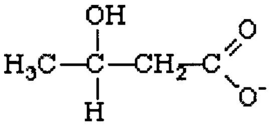
Two new enzymes are required to handle these situations: Enoyl-CoA isomerase (isomerizes a *cis*-3,4-double bond to a *trans*-2,3-double bond), and 2,4-Dienoyl-CoA reductase (reduces the *cis*-4,5-double bond in the *trans*-2,3-*cis*-4,5-dienoyl-CoA derivative formed during *beta*-oxidation). The resulting products are then broken down by the *beta*-oxidation enzymes.

Ketone Bodies Metabolism

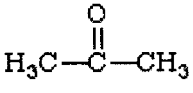
Fatty acids can be used as the major fuel for tissues such as muscle, but they cannot cross the blood-brain barrier, and thus cannot be used by the central nervous system (CNS). This becomes a major problem during starvation (fasting), particularly for organisms such as ourselves in which CNS metabolism constitute a major portion of the resting basal metabolic rate. These organism must provide glucose to the CNS to provide for metabolic needs, and thus during the initial fasting period must break down substantial amounts of muscle tissue (protein) to provide the amino acid precursors of gluconeogenesis. Obviously the organism could not survive long under such a regime. What is needed is an alternate fuel source based on fat rather than muscle. The so-called ketone bodies serve this function:


$H_3C-C(=O)-CH_2-COO^-$

Acetoacetate


$H_3C-CH(OH)-CH_2-COO^-$

D-β-Hydroxybutyrate


$H_3C-C(=O)-CH_3$

Acetone

Note that only two of the ketone bodies are in fact ketones, and that acetone is an "unintentional" breakdown product resulting from the instability of acetoacetate at body temperature. Acetone is **not** available as fuel to any significant extent, and is thus a waste product.

CNS tissues can use ketone bodies any time, the problem is the normally very low concentrations (< 0.3 mM) compared to glucose (about 4 mM). Since the K_M's for both are similar, the CNS doesn't begin to use ketone bodies in preference to glucose until their concentration exceed's the concentration of glucose in the serum.

The system becomes saturated at about 7 mM. The limiting factor in using ketone bodies then becomes the ability of the liver to synthesis them, which requires the induction of the enzymes required for acetoacetate biosynthesis. Normal glucose concentrations inhibit ketone body synthesis, thus the ketone bodies will only begin to be synthesized in high concentrations as serum glucose concentrations fall. As an example, ketone bodies might start at about 0.1 mM after an overnight fast, rise to 3 mM after a 3 day fast, and go to 7-8 mM with prolonged fasting (>24 days).

Ketogenesis (Ketone Synthesis)

Occurs in liver when acetyl CoA production exceeds the limits of its oxidation in the citric acid cycle --------> starvation or uncontrolled diabetes.

Conditions Favouring Ketoacidosis:

Relative or absolute deficiency of insulin
|
V
Mobilization of free fatty acids from fat depots
|
V
Increased delivery of free fatty acids to the liver
|
V
Increased uptake and oxidation of free fatty acids by the liver
|
V
Accelerated production of ketone bodies by the liver

Ketone Body Formation in Liver

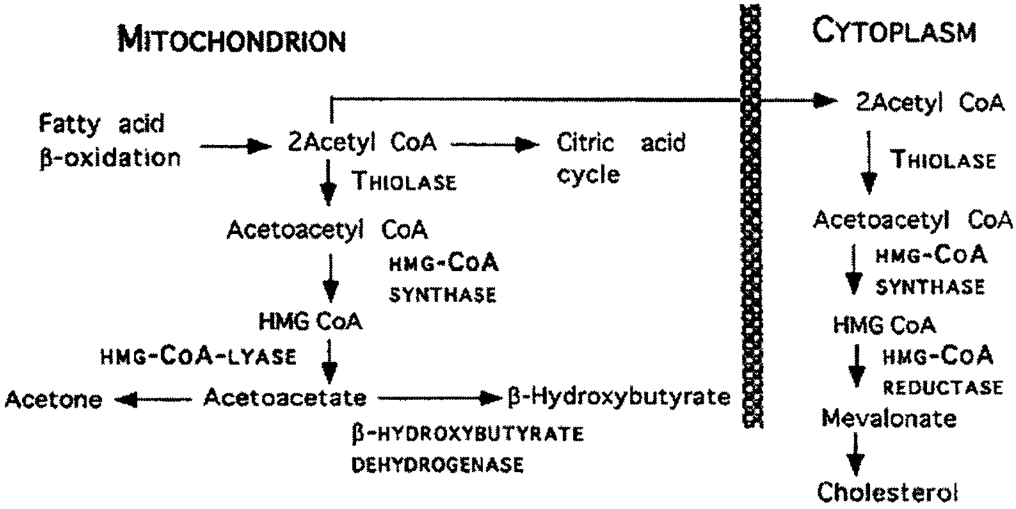
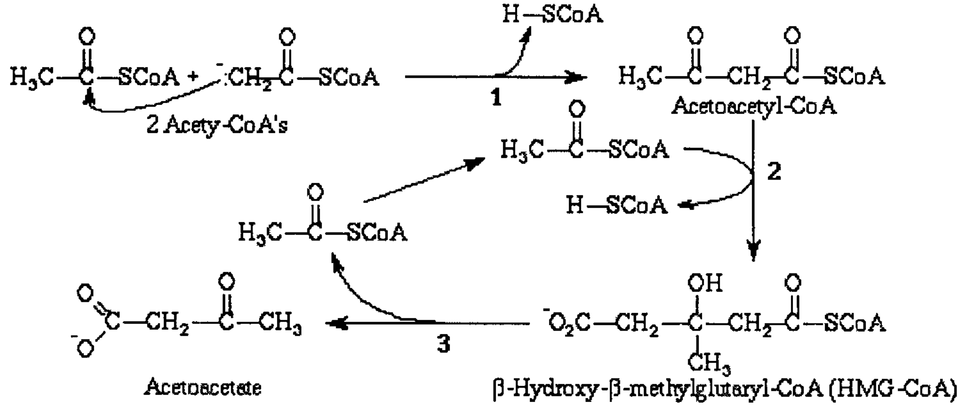

MITOCHONDRION
CYTOPLASM
Fatty acid β-oxidation
2Acetyl CoA
Citric acid cycle
THIOLASE
Acetoacetyl CoA
HMG-CoA SYNTHASE
HMG CoA
HMG-CoA-LYASE
Acetone
Acetoacetate
β-Hydroxybutyrate
β-HYDROXYBUTYRATE DEHYDROGENASE
2Acetyl CoA
THIOLASE
Acetoacetyl CoA
HMG-CoA SYNTHASE
HMG CoA
HMG-CoA REDUCTASE
Mevalonate
Cholesterol

Fig. 10.4 : Ketone Body Formation

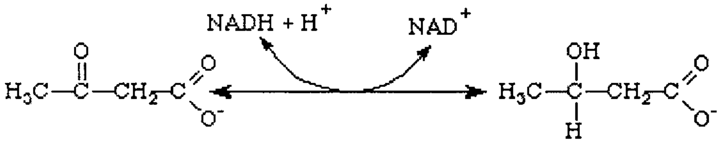
Ketogenesis occurs in the matrix of liver mitochondria. Fatty acids are first broken down to acetyl CoA via *beta*-oxidation (providing energy for liver metabolism from the reducing equivalents generated). The acetyl CoA is then used in ketogenesis:


H₃C–C(=O)–SCoA + ⁻:CH₂–C(=O)–SCoA
2 Acety-CoA's
H–SCoA
1
H₃C–C(=O)–CH₂–C(=O)–SCoA
Acetoacetyl-CoA
H₃C–C(=O)–SCoA
H–SCoA
2
H₃C–C(=O)–SCoA
⁻O₂C–CH₂–C(OH)(CH₃)–CH₂–C(=O)–SCoA
β-Hydroxy-β-methylglutaryl-CoA (HMG-CoA)
3
⁻O(O)C–CH₂–C(=O)–CH₃
Acetoacetate

- The first reaction (1), catalyzed by **thiolase**, involves a Claisen condensation of two acetyl CoA's (essentially a reversal of the last reaction of *beta*-oxidation) to give acetoacetyl CoA - almost the final product! The problem now is to remove the CoASH.

- This is done in a two stage process involving first the addition of a third acetyl CoA by the second enzyme: **HMG-CoA synthase (2)** via an aldol condensation (note the similarity to the formation of citrate in the TCA cycle, including driving the reaction by the hydrolysis of a thiol ester bond).
- The resulting *beta*-Hydroxy-*beta*-glutaryl-CoA is now cleaved by **HMG-CoA lyase (3)**, regenerating acetyl CoA and giving the product: acetoacetate.

Depending on the status of the liver, acetoacetate can now be reduced to give D-*beta*-hydroxybutyrate, which delivers more reducing equivalents, and thus ATP equivalents, to the peripheral tissues at the expense of the liver:



Normal Prevention of Ketoacidosis (Fig. 10.5)

Insulin whose release is promoted by ketone bodies, inhibits lipolysis to decrease the supply of fatty acids and thus curtail ketogenesis ---> prevent ketoacidosis.

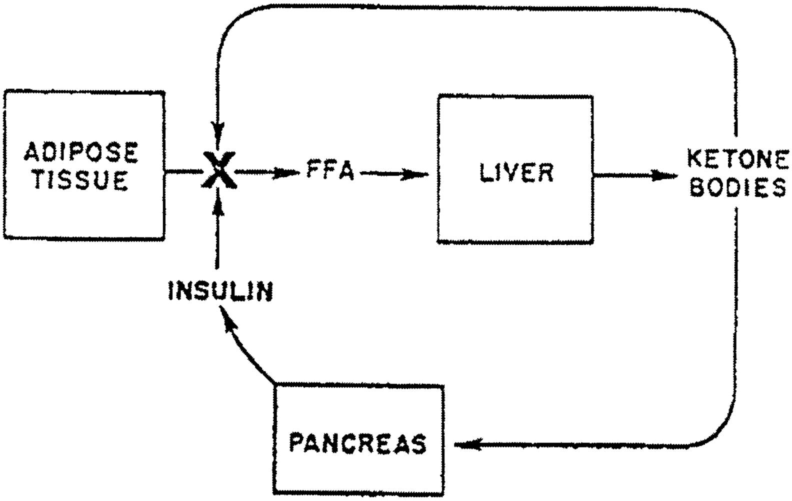


Fig. 10.5 : Prevention of Ketoacidosis

Ketone Body Oxidation: Long-term starvation or ketoacidosis.

Tissues that can use ketones as "fuel": **brain, muscle, kidney, intestine**

β-hydroxybutyrate + NAD^+ ---> NADH + acetoacetate

acetoacetate + succinyl CoA ---> acetoacetyl CoA + succinate

acetoacetyl CoA + CoA ---> 2 acetyl CoA

Ketone Bodies as Fuel

The ketone bodies are water soluble and are transported across the inner mitochondrial membrane as well as across the blood-brain barrier and cell membranes. Thus, they can be used as a fuel source by a variety of tissues including the CNS. They are preferred substrates for aerobic muscle and heart, thus sparing glucose when they are available.

In the peripheral tissues the ketones must be reconverted to acetyl CoA in the mitochondria (Fig. 10.6):

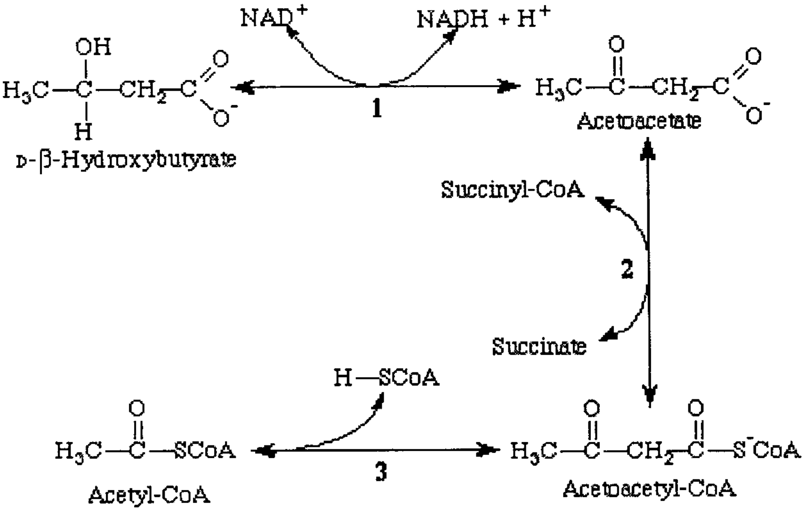


Fig. 10.6 : Acetyl CoA synthesis from Ketone.

- If we start with *beta*-hydroxybutyrate, then it is first oxidized to acetoacetate with the production of one NADH (**1**).
- Coenzyme A must now be added to the acetoacetate. The thioester bond is a high energy bond, so ATP equivalents must be used. In this case the energy comes from a transesterification of the CoAS from succinyl CoA to acetoacetate by Coenzyme A transferase (**2**). The succinyl CoA comes from the TCA cycle where a GTP is **not** made.

- The acetoacetyl CoA is now cleaved to **two acetyl CoA's** with Thiolase (3).

The energy provided to the peripheral tissues from acetoacetate and for *beta*-hydroxybutyrate are shown below: (Table 10.1)

Table 10.1 : Energetics of Ketone Metabolism

Reaction	**Energy Product**	**Factor**	**Multiplier**	**ATP Equiv.**
CoA transferase	succinate (-GTP)	1	1	-1
Kreb's NAD^+ DH's	NADH	2.5 x 3	2	15
Kreb's	GTP	1	2	2
Kreb's FADH DH	$FADH_2$	1.5	2	3
Acetoacetate Total:				**19**
Butyrate DH	NADH	2.5	1	2.5
Butyrate Total:				**21.5**

Note the P/O ratios for the ketone bodies: Acetoacetate = 19 ATP / 8 O = 2.38; Butyrate = 21.5 ATP / 9 O = 2.39 which are higher than we calculated for palmitate (2.3), but again lower than for glucose (2.67).

These reactions can be thought of as giving the liver overall control of fat metabolism. Lower vertebrates store fat in the liver. In a sense adipose tissue can be thought of then as "extended liver" tissue metabolically. Note that the liver can adjust the amount of reducing equivalents, and thus ATP equivalents, it sends to the peripheral tissues by adjusting the amounts of acetoacetate vs. *beta*-hydroxybutyrate it exports. Thus, the percentage of the free energy distributed between the tissues is shown below as Table 10.2 :

Table 10.2 : Free Energy Distribution between Tissues during FAT Metabolism

Compound \ Tissue	**Liver**	**Peripheral Tissues**
3-hydroxybutyrate	17%	83%
Acetoacetate	26%	74%

Fatty Acid Biosynthesis

Earlier, it was thought that fatty acid biosynthesis occurred by reversal of the β-oxidation pathway. On the contrary, it occurs by a separate pathway that differs from β-oxidation in several ways.

- Synthesis takes place in the cytosol, in contrast with degradation or oxidation, which occurs in the mitochondrial matrix.
- Intermediates in fatty acid synthesis are covalently linked to the sulphhydryl group of an acyl carrier protein (ACP) whereas intermediates in fatty acid breakdown are bonded to coenzyme A.
- The enzymes of fatty acid synthesis in animals are joined in a single polypeptide chain called fatty acid synthase. In contrast, the degradative enzymes do not seem to be associated. Plants employ separate enzymes to carry out the biosynthetic reactions.
- The reductant in fatty acid synthesis is NADPH, whereas the oxidants in fatty acid oxidation are NAD+ and FAD.

Lipogenesis (Fatty Acid Synthesis)

Pyruvate-Malate Cycle

The reactions of fatty acid synthesis all take place in the cytosol, but acetyl-CoA is made in the mitochondria and can't cross the inner membrane. The **Pyruvate-Malate Cycle** (Citrate-Pyruvate Cycle) is used to take acetyl- groups to the cytosol while simultaneously providing a source of NADPH from NADH, and thus, coupling fatty acid synthesis to Glycolysis (Fig. 10.7). Note that the acetyl-CoA is first joined to oxaloacetate to make citrate which is readily transported out of the mitochondria using a co-transporter. The citrate is then cleaved to acetyl-CoA and oxaloacetate, a process requiring ATP to make it favourable (recall the condensation was spontaneous). Acetyl-CoA for fatty acid synthesis is now available in the cytosol, but oxaloacetate must be regenerated for the mitosol.

The cytosolic oxaloacetate is now dehydrogenated to give malate and NAD^+. Malate is next oxidized by **Malic enzyme** to give pyruvate in a reaction which also provides NADPH for use in biosynthesis. (Note that NADH generated in Glycolysis is "converted" to NADPH for Fatty Acid synthesis in these two reactions, while simultaneously regenerating the NAD^+ needed to continue Glycolysis!) The pyruvate can now cross into the mitosol to be used in regenerating oxaloacetate.

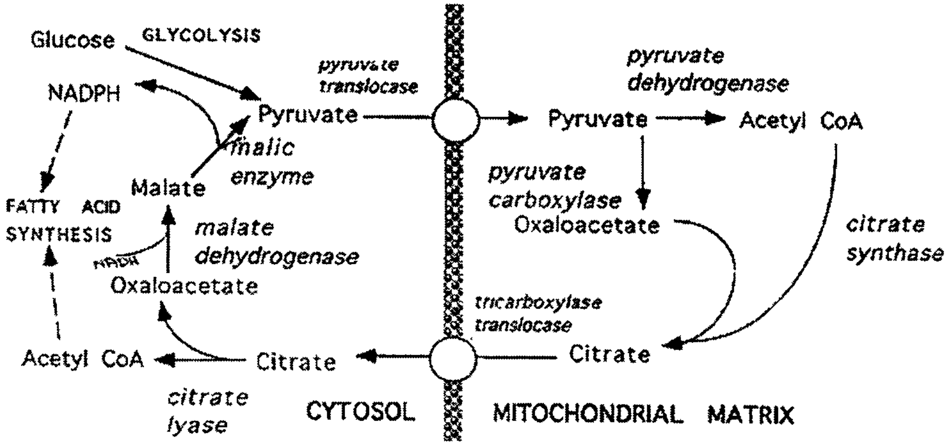

Glucose
GLYCOLYSIS
NADPH
Pyruvate
malic enzyme
Malate
FATTY ACID SYNTHESIS
malate dehydrogenase
NADH
Oxaloacetate
Acetyl CoA
Citrate
citrate lyase
pyruvate translocase
tricarboxylase translocase
CYTOSOL
pyruvate dehydrogenase
Pyruvate
Acetyl CoA
pyruvate carboxylase
Oxaloacetate
citrate synthase
Citrate
MITOCHONDRIAL MATRIX

Fig. 10.7 : Export of Acetyl CoA for Fatty Acid Biosynthesis

$$\text{AcetylCoA}_{mit} + 2ATP + NADH + NADP^{+} \dashrightarrow \text{AcetylCoA}_{cyt} + 2ADP + 2P_{i} + NAD^{+} + NADPH$$

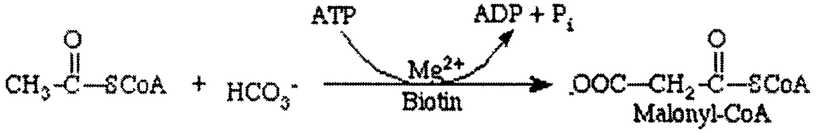
Now, let fatty acid biosynthesis starting from glucose. For that look at the integration of the various pathways involved: Glycolysis, hexose monophosphate shunt, Pyruvate-malate shuttle, and Fatty acid biosynthesis. It requires reducing equivalents, redox balance and provision of required cytosolic ATP's as well as carbon source.

The following steps are involved in fatty acid biosynthesis in cytosol

Two reactions enable the synthesis pathway:

- Acetyl-CoA is "activated" by the addition of a carbon dioxide, and
- NADPH is substituted as a more powerful reducing agent than $FADH_2$.

Thus, the first step in fatty acid biosynthesis is to activate acetyl-CoA by the addition of a carbon dioxide using **Acetyl-CoA carboxylase**. This reaction is chemically identical to the Pyruvate carboxylase reaction.



$$CH_3-\overset{\overset{O}{\|}}{C}-SCoA + HCO_3^- \xrightarrow[\text{Biotin}]{ATP \quad Mg^{2+} \quad ADP + P_i} .OOC-CH_2-\overset{\overset{O}{\|}}{C}-SCoA$$

Malonyl-CoA

This reaction is physiologically irreversible and is the flux generating or first committed step of fatty acid biosynthesis. As expected it is regulated. In mammals acetyl-CoA carboxylase is a large enzyme existing as inactive protomers (560,000 MW, 4 subunits, one biotin), which can assemble into active filaments (4 - 10 million MW).

Formation of the filaments (activation) is :

- Promoted by citrate, but
- Inhibited by fatty acyl CoA.

As these are excellent indicators of the fuel status of the cell.

In addition to the activator/inhibitor controls of citrate and fatty acyl-CoA, the enzyme is also under hormonal control (note the similarities to glycogen control):

- Glucagon stimulates phosphorylation and thus inactivation of the liver enzyme, while
- Adrenalin (epinephrin) stimulates phosphorylation and inactivation in adipose tissue.

In mammals **Fatty Acid Synthase (FAS)** catalyzes fatty acid synthesis on a homodimeric enzyme, each monomer of which has **seven catalytic activities**, and **eight sites**! (In bacteria such as *E. coli* there are seven separate enzymes plus an acyl-carrier protein. Plants also have individual proteins for the various activities which are associated in a quaternary complex. In eukaryotes other than plants the FAS are complexes of multifunctional proteins. The enzyme weighs approximately 500,000 Daltons.

[*Reference*: Maier Timm, Simon Jenni and Nenad Ban. "Architecture of Mammalian Fatty Acid Synthase at 4.5 A Resolution." *Science* **311**(3 March 2006) 1258; mini review comparing fungal and mammalian enzymes - Smith, Stuart. "Architectural Options for a Fatty Acid Synthase." *Science* **311**(3 March 2006) 1251.]

There are two carriers on this complex.

- The first is referred to as ACP_1 which acts as a holding station for acetyl- or fatty acyl- groups. In either case they are bound to a cysteinyl sulphhydryl group.
- The second, ACP_2, binds the growing fatty acyl chain during the condensation and reduction reactions of the cycle. In this case the acyl group is carried on a long phosphopantetheine prosthetic group. This

arm allows the growing chain to move among the various active sites without being lost to the solution.

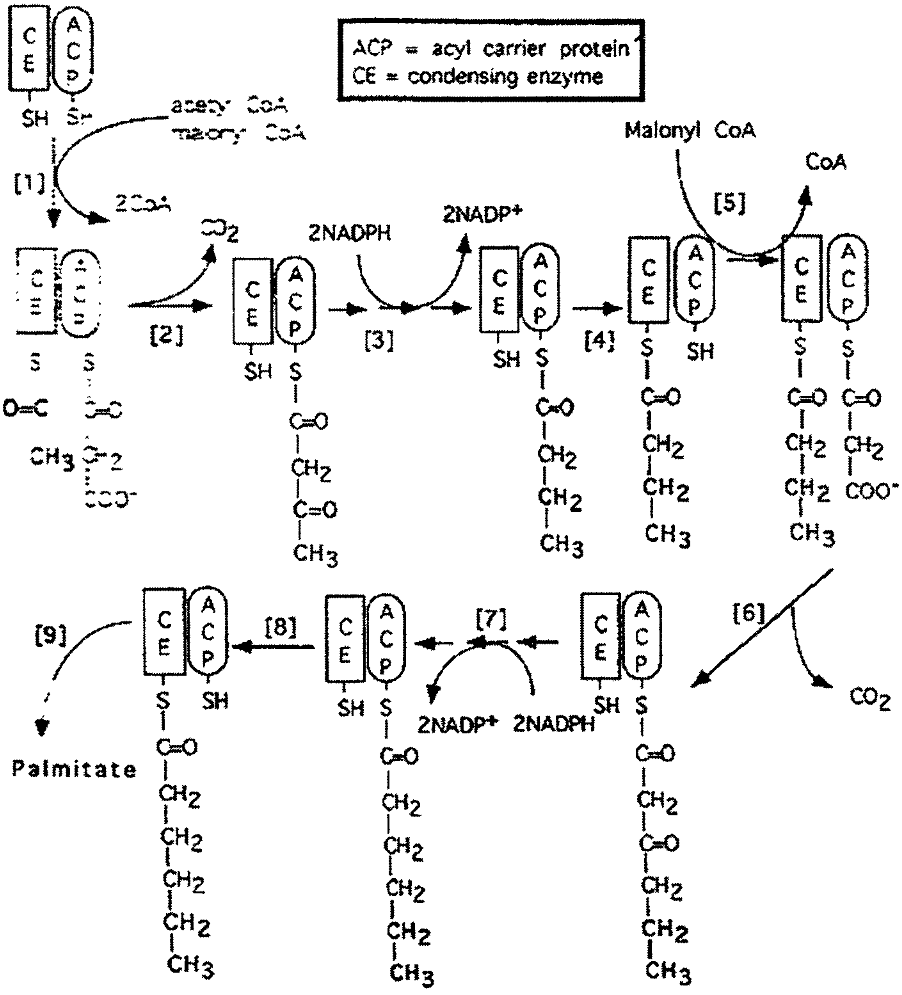

ACP = acyl carrier protein
CE = condensing enzyme
C E
A C P
SH
acetyl CoA
malonyl CoA
[1]
2CoA
CO2
[2]
2NADPH
[3]
2NADP+
[4]
Malonyl CoA
[5]
CoA
[6]
CO2
[7]
2NADP+ 2NADPH
[8]
[9]
Palmitate
S
C=O
CH2
CH3
COO-

Fig. 10.8 : Fatty Acid Synthesis Reactions

Looking at the the **Fatty Acid Synthase reactions** (Fig. 10.8)

The first reaction, catalyzed by **Acetyl-CoA:ACP transacylase**, transfers an acetyl group from Coenzyme A to the cysteinyl-S on ACP_1.

- Next, a malonyl-group is transferred from a Coenzyme A to the pantetheinyl-S of ACP_2 by **Malonyl-CoA:ACP transacylase.**

- Now the carbon dioxide leaves the malonyl group, with the electrons from its bond attacking the acyl group on ACP_1. This reaction is catalyzed by **Ketoacyl-ACP synthase.**

Now have a *beta*-ketoacyl group ready to go through the reverse of the reactions of *beta*-oxidation.

- Thus, the keto-group is reduced to an alcohol using NADPH (***beta*-ketoacyl-ACP reductase**),
- followed by the elimination of the alcohol (**Enoyl-ACP hydrase**) to give the *cis*-2,3-enoyl group.
- The enoyl is then reduced with NADPH substituting for $FADH_2$ (**Enoyl-ACP reductase**) to give the saturated acyl group.
- Finally the acyl group is transferred from the pantotheinyl-S of ACP_2 to the cysteinyl-S on ACP_1 (**ACP-acyltransferase**) leaving ACP_2 available to pick up the next malonyl moiety.
- After seven turns of the cycle palmitate is released.
- Elongation by the fatty acid synthase complex stops upon formation of palmitate (16 C). Further elongation and the formation of double bonds are carried out by other enzyme systems.

Summary of Fatty Acid Biosynthesis

$$\text{Acetyl CoA} + 7\ \text{malonyl CoA} + 14\ \text{NADPH} + 20\ H^+ \longrightarrow \text{Palmitate} + 7\ CO_2 + 14\ \text{NADP}^+ + 8\ \text{CoASH} + 6\ H_2O$$

The equation for the synthesis of the malonyl CoA used in the above reaction is

$$7\ \text{Acetyl CoA} + 7\ CO_2 + 7\ \text{ATP} \longrightarrow 7\ \text{malonyl CoA} + 7\text{ADP} + 7\ \text{Pi} + 14\ H^+$$

The overall reaction for the synthesis of palmitate is

$$\mathbf{8\ Acetyl\ CoA + 7\ ATP + 14\ NADPH + 6H^+ \longrightarrow Palmitate + 14\ NADP + 8\ CoASH + 6\ H_2O + 7\ ADP + 7\ Pi}$$

Fatty acid synthesis and degradation are reciprocally regulated so that both are not simultaneously active.

Regulation of Lipogenesis

Enzyme		Regulatory agent	Effect
Acetyl CoA carboxylase	**Short-term**	Insulin	Stimulation
		Glucagon	Inhibition
	Long-term	High-carbohydrate, low-fat diet	↑ enzyme synthesis
		High-fat diet	↓ enzyme synthesis
		Fasting	↓ enzyme synthesis
Fatty acid synthase		High-carbohydrate, low-fat diet	↑ enzyme synthesis
		High-fat diet	↓ enzyme synthesis
		Fasting	↓ enzyme synthesis

Conditions Favoring Lipogenesis

increased glucokinase activity in liver - *increases metabolism of glucose as a fatty acid precursor*

decreased fatty acid availability - *reduces inhibition of lipogenesis*

activation of acetyl CoA carboxylase - *increases production of malonyl CoA*

increased flux through pentose shunt - *produces NADPH for fat synthesis*

hypercaloric high carbohydrate or high protein, low-fat diet - *provides precursors for fat synthesis*

Comparison of Fatty Acid β-Oxidation and Synthesis

Parameter	Oxidation	Synthesis
Intracellular location	Mitochondria	Cytoplasm
Coenzymes	FAD, NAD^+	NADPH
Bicarbonate dependence	No	Yes
Citrate activation	No	Yes
Acyl CoA inhibition	No	Yes
Malonyl CoA inhibition	Yes	No
Highest activity	Fasting	Carbohydrate fed

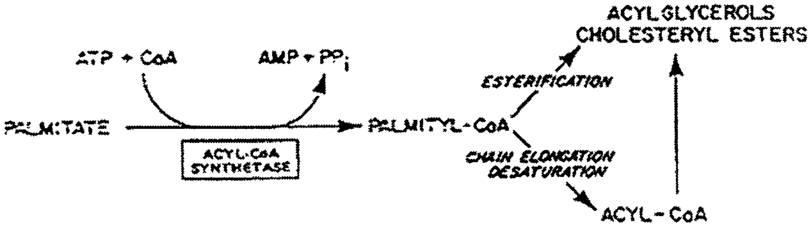
Fatty Acid Modification

Elongation of Fatty Acids

If the Fatty Acid Synthetase Complex only makes palmitate where do the rest of the fatty acids come from? Of course palmitate can be shortened by β-oxidation. For longer fatty acids there is a fatty acid elongation system localized on the ER. The same reactions occur as in the Synthetase, but now have individual enzymes. Palmitate is first activated to palmitoyl-CoA. The enzymes prefer C-16 or less as substrate; thus the major product is stearoyl-CoA. However longer unsaturated fatty acids will also bind (the linking of the *cis* double bond makes them effectively shorter), so unsaturated fatty acids of 20, 22, and 24-C's are also made. Thus most longer fatty acids are polyunsaturated.

A second system for fatty acid elongation exists in the mitosol, probably for provision of long fatty acids for mitochondrial structure. This system uses most of the same activities of β-oxidation, but an NADPH dependent Enoyl-CoA reductase replaces the FAD dependent dehydrogenase.

Acyl-CoA Synthetase



Elongation: microsomal process (ER), requires malonylCoA and two NADPH, process is similar to that occurring with fatty acid synthase.

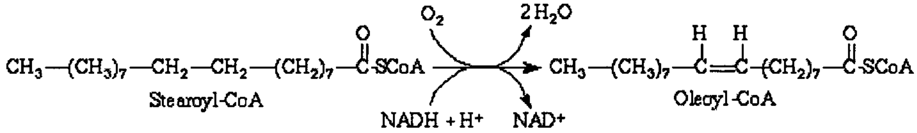
Fatty Acid Desaturation

Plants and animals differ in where double bonds are introduced into fatty acids.

Plants put in Δ^9 (a 9-10 double bond) and then can put in double bonds at three carbon intervals towards the tail (Δ^{12}, Δ^{15}). They can also add Δ^6, but not common.

Animals also start with Δ^9, then can add at three carbon intervals toward carboxy end (Δ^6 and Δ^6). Animals cannot add towards the tail. Therefore animals cannot make linoleoyl-CoA ($\Delta^{9, 12}$; C18) but can make oleoyl-CoA (Δ^9; C18). Animals must therefore take in plant products (either directly as herbivores, or indirectly by eating herbivores) to acquire essential unsaturated fatty acids such as linoleic and arachadonic acids.

Both plants and animals use **mixed function oxidases** (simultaneously oxidize two substrates): **Acyl-CoA desaturases** localized on the ER. Similar mixed function oxidases are also used to modify structural components of cells, hormones etc. so we will use the acyl-CoA desaturase as an example for this group of enzymes. In the acyl-CoA desaturase reaction molecular oxygen is used to oxidize both a fatty acid and NADH, each providing two of the the four electrons needed by the oxygen:


O₂
2H₂O
CH₃—(CH₃)₇—CH₂—CH₂—(CH₂)₇—C(=O)-SCoA
Stearoyl-CoA
NADH + H⁺
NAD⁺
CH₃—(CH₃)₇—CH=CH—(CH₂)₇—C(=O)-SCoA
Oleoyl-CoA

The mammalian acyl desaturases are components in mini-electron transport systems on the surface of the endoplasmic reticulum, for example the Δ^9-fatty acyl-CoA desaturase complex:

Unsaturation: microsomal process, reduction with NADPH or NADH, molecular oxygen is required.

Triacylglycerol Biosynthesis (Esterification) (Fig. 10.9)

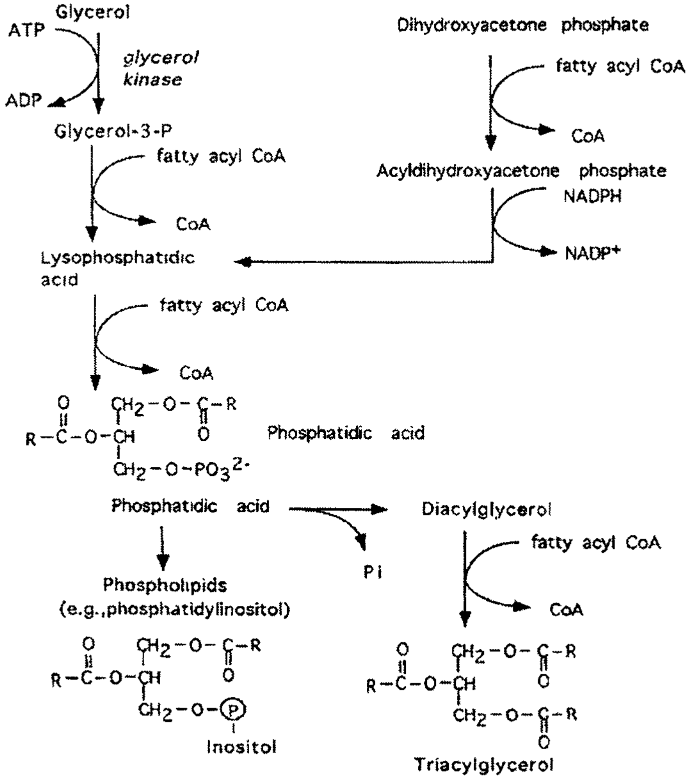

Glycerol
ATP
ADP
glycerol kinase
Glycerol-3-P
fatty acyl CoA
CoA
Lysophosphatidic acid
fatty acyl CoA
CoA
Phosphatidic acid
Dihydroxyacetone phosphate
fatty acyl CoA
CoA
Acyldihydroxyacetone phosphate
NADPH
NADP⁺
Phosphatidic acid
Diacylglycerol
Pi
Phospholipids (e.g.,phosphatidylinositol)
fatty acyl CoA
CoA
Inositol
Triacylglycerol

Fig. 10.9 : Formation of Phosphatidic Acid from Glycerol or Dihydroxyacetone Phosphate and its Conversion to Triacylglycerol or Phospholipids.

Regulation of Fatty Acid Metabolism

Fatty acid metabolism is regulated both hormonally and via feed-back inhibition and feed-forward activation. Thus mobilization of free fatty acids from the adipose tissue results from low insulin levels. The free fatty acids are then transported through the blood to the rest of the body including the liver. In the liver fatty acid oxidation and ketone body synthesis is activated by glucagon. Note that glucagon and insulin levels are opposite: high insulin =low glucagon and vice-versa. So for low insulin will also have high glucagon, thus fatty acids will be released from the adipose and will be converted in the liver into ketone bodies.

The regulation of fatty acid oxidation, fatty acid synthesis and ketone body synthesis in the liver is summarized in fig. 10.10:

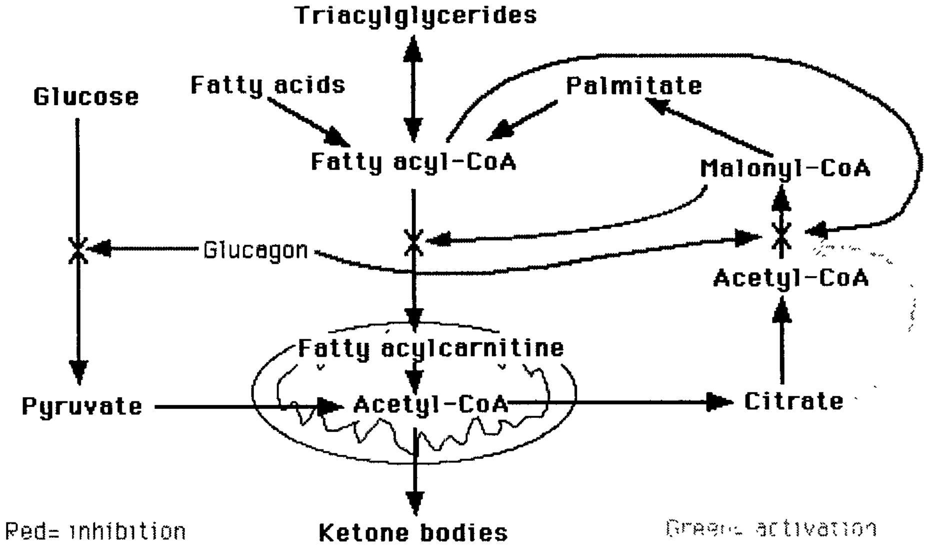
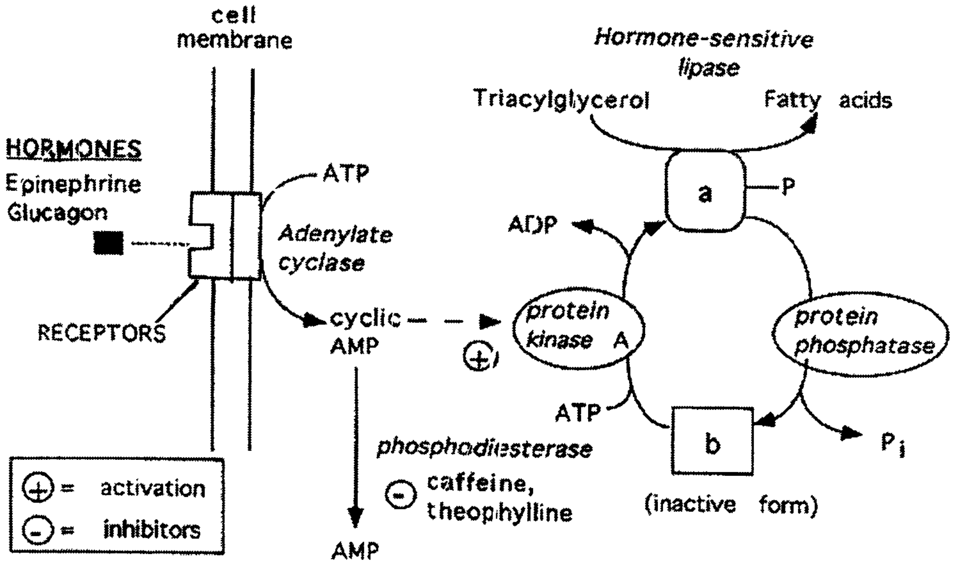

Triacylglycerides
Glucose
Fatty acids
Palmitate
Fatty acyl-CoA
Malonyl-CoA
Glucagon
Acetyl-CoA
Fatty acylcarnitine
Pyruvate
Acetyl-CoA
Citrate
Ketone bodies
Red= inhibition
Green= activation

Fig. 10.10 : Regulatory Interations of Fatty Acid Synthesis and Oxidation in Liver

Note that a lack of insulin results in a release of fatty acids from adipose.

Regulation of Hormone-Sensitive Lipase (Lipolysis) : "Fasted" State (Fig. 10.11)


cell membrane
HORMONES
Epinephrine
Glucagon
RECEPTORS
ATP
Adenylate cyclase
cyclic AMP
phosphodiesterase
caffeine, theophylline
AMP
Hormone-sensitive lipase
Triacylglycerol
Fatty acids
a
P
ADP
protein kinase A
protein phosphatase
ATP
b
(inactive form)
P_i
⊕ = activation
⊖ = inhibitors

Phosphorylated- *Active*

Dephosphorylated- *Inactive*

Fig. 10.11 : Lipolysis at Fasted State

Regulation of Acetyl CoA Carboxylase (Lipogenesis): "Fed" State (Fig. 10.12)

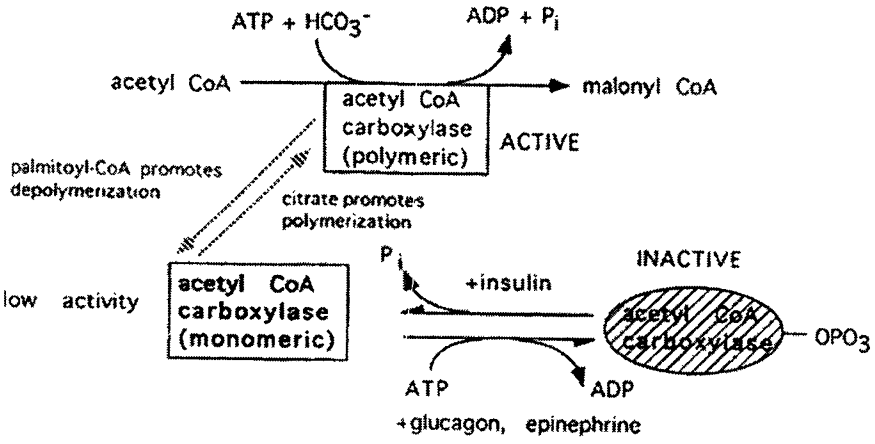

ATP + HCO_3^-
ADP + P_i
acetyl CoA
malonyl CoA
acetyl CoA carboxylase (polymeric)
ACTIVE
palmitoyl-CoA promotes depolymerization
citrate promotes polymerization
low activity
acetyl CoA carboxylase (monomeric)
P_i
+insulin
INACTIVE
acetyl CoA carboxylase
OPO_3
ATP
ADP
+glucagon, epinephrine

Fig. 10.12 : Lipogenesis at Fed State

Allosteric Control:

Polymer- *Active*

Monomer- *Inactive*

Citrate ----> Polymerization ----> "Active" (lipogenesis)

Palmitoyl CoA ----> Depolymerization ----> "Inactive"

Covalent Modification:

Glucagon, Epinephrine (low glucose) ----> Phosphorylation ----> "*Inactive*" (while promoting lipolysis)

Insulin ----> Dephosphorylation ----> "*Active*"

Induction / Repression: (Fig. 10.13)

High CHO and Low Fat Diets ----> "*Inductive*" (increased synthesis)

High Fat Diet and Fasting ----> "*Repression*" (decreased synthesis)

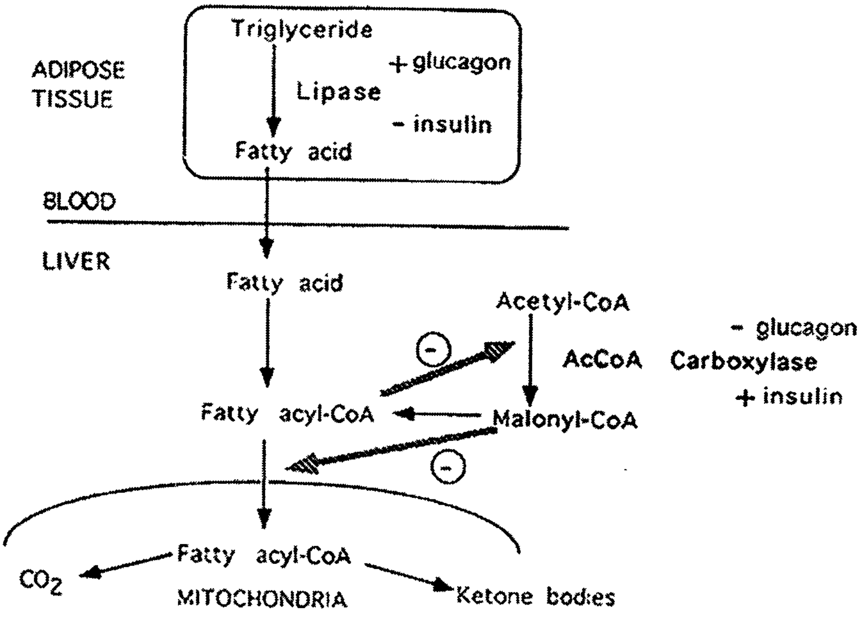

ADIPOSE TISSUE
Triglyceride
Lipase
+ glucagon
- insulin
Fatty acid
BLOOD
LIVER
Fatty acid
Acetyl-CoA
AcCoA Carboxylase
- glucagon
+ insulin
⊖
Fatty acyl-CoA
Malonyl-CoA
⊖
Fatty acyl-CoA
CO_2
MITOCHONDRIA
Ketone bodies

Fig. 10.13 : Coordinated Control of Fat Synthesis and Breakdown via Acetyl CoA and Malonyl CoA

Interrelationship of Fat and CHO Metabolism When Glucose is High: (Fig. 10.14)

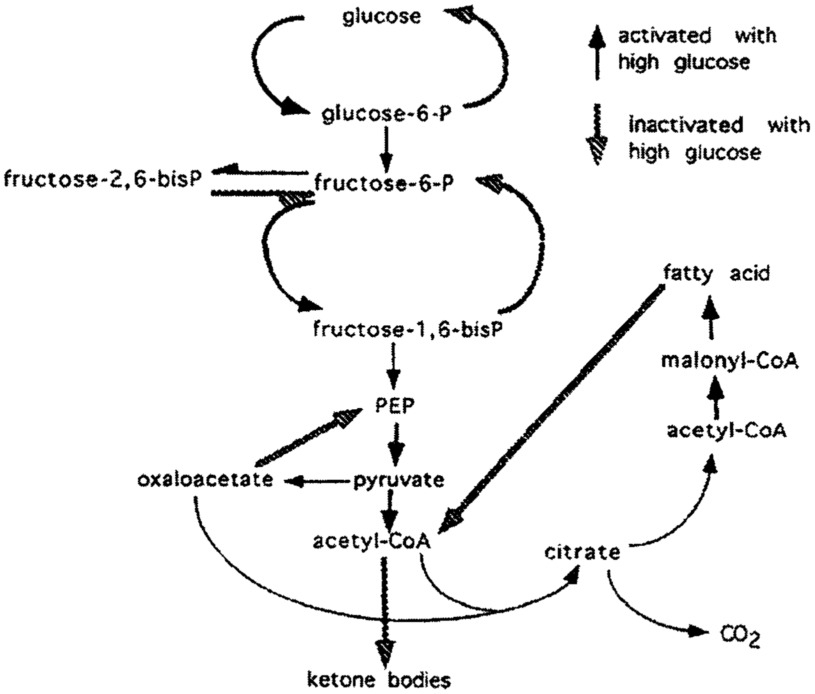

glucose
glucose-6-P
fructose-2,6-bisP
fructose-6-P
fructose-1,6-bisP
PEP
oxaloacetate
pyruvate
acetyl-CoA
ketone bodies
citrate
CO2
fatty acid
malonyl-CoA
acetyl-CoA
activated with high glucose
inactivated with high glucose

Fig. 10.14 : Fat and CHO Metabolism at high Glucose Level

Conditions Favoring Fat Synthesis (*Lipogenesis*):

CHO intake ----> Elevated Blood Glucose ----> Insulin High, Glucagon Low

Insulin (-) hormone sensitive lipase; (+) glucose utilization (glycolysis) and acetyl CoA production for lipogenesis.

Citrate (+) acetyl CoA carboxylase.

Malonyl CoA (-) carnitine palmitoyl transferase I (decreasing β-oxidation).

Conditions Favoring Lipolysis / β-*Oxidation*:

Starvation ----> Low Blood Glucose ----> Insulin Low, Glucagon High ----> (+) Lipolysis (free fatty acids for liver).

"Fight or Flight" ----> Epinephrine High ----> (+) Lipolysis (energy for muscle).

Low Blood Glucose ----> (+) gluconeogenesis (decreasing "C" supply for lipogenesis).

Acetyl CoA Carboxylase- phosphorylated ("Inactive").

CPT I- "Active" (due to decrease in Malonyl CoA).

Interrelationship of Fat and CHO Metabolism When Glucose is Low: (Fig. 10.15)

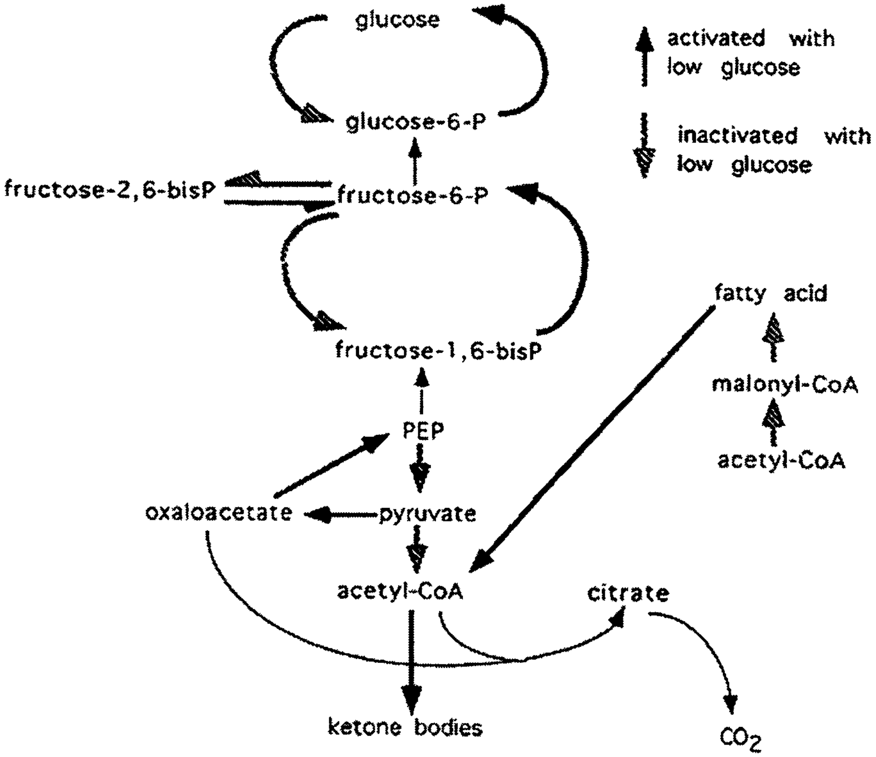

glucose
glucose-6-P
fructose-2,6-bisP
fructose-6-P
fructose-1,6-bisP
PEP
oxaloacetate
pyruvate
acetyl-CoA
citrate
ketone bodies
CO_2
activated with low glucose
inactivated with low glucose
fatty acid
malonyl-CoA
acetyl-CoA

Fig. 10.15 : Fat and CHO Metabolism at low Glusose Level

Starvation:

Fatty Acids are Oxidized in Liver which Promotes Gluconeogenesis by:

(a) Providing energy.

(b) Generating NADH.

(c) Forming acetyl CoA to activate pyruvate carboxylase.

(d) Producing citrate which increases F-1,6 bisPase activity.

Comparison of **Energy Yields** and **Oxygen Consumption**:

1 NADH = 3 ATP

1 $FADH_2$ = 2 ATP

Palmitate (3 molecules = 48 "C"s)

Step 1: β-oxidation to acetyl CoA (6 cycles); (1 NADH + 1 ADH2)/cycle

Step 1cont'd: 7 x 3 molecules 21 FADH2+ 21 NADH ==> +105 ATP;
- 42 O atoms

Step 2: Acetyl CoA oxidation via TCA cycle;
(3 NADH, 1 FADH2, 1 GTP) / Acetyl CoA

Step 2 cont'd: 8(6 + 2) x 3 molecules: 24 Acetyl CoA ==> +288 ATP;
-96 O atoms

Step 3: Acyl CoA formation (ATP --> AMP + PPi; 2 ATP / molecule)

Step 3 cont'd: 2 x 3 molecules: ==> - 6 ATP

β-hydroxybutyrate (12 molecules = 48 "C"s) Ketone

Step 1: oxidation to acetoacetate via b-hydroxybutyrate DH

Step 1 cont'd: 1 x 12 molecules: 12 NADH ==> +36 ATP; -12 O atoms

Step 2: Acetoacetate cleaved to 2 acetyl CoA (loss 1 GTP due to succinyl CoA diversion)

Step 2 cont'd: 1 x 12 molecules: -12 GTP ==> -12 ATP

Step 3: Oxidation of acetyl CoA via TCA cycle

Step 3 cont'd: 2 x 12 molecules: 24 Acetyl CoA ==> +288 ATP;
-96 O atoms

Glucose (8 molecules = 48 "C"s)

Step 1: Aerobic glycolysis, 2 NADH (mal-asp shuttle) + 2 ATP/glucose

Step 1 cont'd: 2 x 8 molecules: 16 NADH + 16 ATP ==> +64 ATP;
- 16 O atoms

Step2: PDH

Step 2 cont'd: 2 x 8 molecules: 16 NADH ==> +48 ATP; -16 O atoms

Step 3: Oxidation of acetyl CoA via TCA cycle

Step 3 cont'd: 2 x 8 molecules: 16 Acetyl CoA ==> +192 ATP; - 64 O atoms

Table 10.3 : Comparises of Energy yields and Oxygen consumption of Different Molecules

Fuel	Total ATP	O_2 Used	ATP/"C"	CO_2/O_2	ATP/"O" Atom
Palmitate	387	69	8.12	0.7	2.80
Ketone	312	54	6.50	0.9	2.89
Glucose	304	48	6.33	1.0	3.17

Note: ATP / "O" Atom is a measure of "fuel" efficiency

Fats: Produce more ATP per "C", however it is at the expense of more oxygen (ATP/ O atom), thus the respiratory quotient (CO_2 / O_2) is the lowest. (Table 10.3)

Glucose: is the better fuel under conditions of O_2 limitation, giving more ATP/ O atom. (Table 10.3)

Ketones: in starvation increase the amount of O_2 needed to burn fuel to CO_2 (CO_2 / O_2) only a small amount as compared to fats. The energy yield per carbon for ketones is similar to glucose. The brains energy needs and O_2 availability can be met nearly as well by ketones, as by glucose. (Table 10.3).

QUIZ

Identify the letter of the choice that best completes the statement or Answers the question.

1. Long-chain fatty acids are oxidized step-wise in ___ carbon units starting from the ___ end.
   A. aliphatic
   B. carboxyl
   C. either
   D. none

Answer : B

2. The irreversibility of the thiokinase reactions (formation of acyl-CoA)
   A. is due to the subsequent hydrolysis of the PP_i product.

B. is shown by the large -DG°' for the reaction.
C. applies only to even-chain fatty acids.
D. none

Answer : A

3. The acyl-CoA formed in the cytosol is transported to the ___ for oxidation using a shuttle involving the intermediate formation of acyl- _____.
A. mitochondrial matrix, carnitine.
B. mitochondrial matrix, coenzyme A.
C. endoplasmic reticulum, albumin.
D. endoplasmic reticulum, carnitine.

Answer : A

4. Each cycle of b-oxidation produces
A. 1 FAD, 1 NADH, and 1 acetyl-CoA.
B. 1 $FADH_2$, 1 NADH, and 1 acetyl-CoA.
C. 1 FAD, 1 NAD^+, and 2 CO_2 molecules.
D. 1 $FADH_2$, 1 NADH, and 2 CO_2 molecules.

Answer : B

5. The last step in b-oxidation (thiolysis) has features that are similar to those of the serine proteases:
A. nucleophilic attack by a Ser or Cys residue.
B. formation of an acyl-enzyme intermediate.
C. specificity for positively charged substrate.
D. The first and second choices are both correct.

Answer : D

6. Oxidation of palmitic acid (C_{16}) involves ___ rounds of b-oxidation and yields ___ molecules of acetyl-CoA.
A. 8, 8
B. 7, 8
C. 16, 8
D. 7, 7

Answer : B

7. Which of the following would yield the most energy per gram when oxidized?
A. starch.
B. glycogen.
C. fat.
D. protein.

Answer : C

8. A general process that breaks down large molecules into smaller ones is called:
A. catalysis.

B. metabolism.
C. dehydration.
D. catabolism.

Answer : D

9. Regulated metabolic pathways are:
A. irreversible.
B. committed after the first step.
C. usually regulated at the first step.
D. All of the above are correct.

Answer : D

10. Each reaction in a metabolic pathway is:
A. controlled by the end product.
B. catalyzed by a specific enzyme.
C. irreversible.
D. reversible.

Answer : B

11. Activation of the ___ kinase results in the activation of ___ kinase and thereby the phosphorylation of both glycogen phosphorylase and glycogen synthase.
A. pyruvate phosphorylase
B. phosphorylase cAMP-dependent protein
C. cAMP-dependent protein phosphorylase
D. cAMP-dependent protein pyruvate

Answer : C

12. When it functions as a "second messenger", cAMP
A. acts outside the cell to influence cellular processes.
B. acts "second in importance" to AMP.
C. activates all cytosolic protein kinases.
D. activates the cAMP-dependent protein kinase.

Answer : D

13. The major free energy sources for anabolic pathways are:
A. ATP and NADPH.
B. ATP and $NADP^+$.
C. ADP and NADPH.
D. ADP and $NADP^+$.

Answer : A

14. Transfer of fatty acids from the cytoplasm to the intra-mitochondrial space involves which of the following?
A. choline
B. 3-hydroxy-4-trimethylamine-lysine
C. carnitine

D. phosphoarginine

Answer: C

15. Unbranched fatty acids with an odd number of carbon atoms are usually converted after b-oxidation to:

A. acetyl-CoA only
B. acetoacetyl-CoA + acetyl-CoA
C. b -methylbutyryl-CoA + acetyl-CoA
D. propionyl-CoA + acetyl-CoA

Answer: D

16. Oxidation of the following unsaturated fatty acid:
H3C(CH2CH2)CH=CHCH2CH=CH(CH2CH2)3CH2CO-SCoA

A. involves the utilization of an epimerase at the first double bond (D 9,10) and the action of an isomerase at the second double bond (D 12,13)
B. involves the utilization of an epimerase at the first (D 9,10) and the second (D 12,13)
C. involves the utilization of an isomerase at the first double bond (D 9,10) and the action of an epimerase at the second double bond (D 12,13)
D. involves the utilization of an isomerase at the first (D 9,10) and the second (D 12,13) double bonds

Answer: C

17. In eucaryotic cells beta oxidation of fatty acids occurs in the

A. cytoplasm
B. inner membrane
C. mitochondrial matrix
D. peroxisome

Answer: C

18. What are the basic building blocks during biosynthesis of fatty acids?

A. three-carbon units
B. two-carbon units
C. two-nitrogen units
D. glucose molecules

Answer: B

19. Which of the following is the reducing agent for fatty acid biosynthesis?

A. FADH2
B. NADH
C. NADPH
D. all of the above

Answer: C

20. During fatty acid biosynthesis, the eukaryotic cell requires a lot of acetyl-CoA. Where does the cell get most of the required acetyl-CoA?
    A. from the nucleus
    B. from the mitochondria
    C. from the golgi apparatus
    D. all of the above

Answer: B

21. Which of the following is considered a committed step in fatty acid biosynthesis?
    A. the production of acetate
    B. the production of malonyl CoA
    C. the transcarboxylation of lysine
    D. all of the above

Answer: B

22. Which of the following uses a single multienzyme complex to accomplish fatty acid biosynthesis?
    A. Escherichia coli
    B. human
    C. Bacillus stearothermophilus
    D. all of the above

Answer: B

23. Which of the following is NOT a type of complex lipid?
    A. sphingolipid
    B. phospholipid
    C. palmitate
    D. All of the above are complex lipids.

Answer: C

24. Which of the following are NOT intermediates of phosphatidic acid biosynthesis?
    A. glycerol-3-phosphate
    B. 1-acyldihydroxyacetone-phosphate
    C. 1-acylglycerol-3-phosphate
    D. glucose

Answer: D

25. How many mevalonate molecules are needed to make one squalene?
    A. 1
    B. 2
    C. 4
    D. 6

Answer: D

26. What is the most common cause of the elevated levels of serum cholesterol in familial hypercholesterolemia?
    A. Too much cholesterol is produceD.
    B. The cholesterol is absorbed into cells too fast.
    C. The cholesterol is absorbed into cells too slowly or not at all.
    D. None of the above

Answer: C

27. In the pathway that produces sphingomyelin from ceramide, what compound is converted to 1,2-diacylglycerol in the process?
    A. phosphatidylcholine
    B. N-acetylgalactosamine
    C. sialic acid
    D. galactose

Answer: A

28. Oxidation of palmitate produces how many net ATPs ?
    A. 124 B. 136 C. 106 D. 152

Answer: C

29. ____________ produce more ATP per carbon and having lowest respiratory quotient.
    A. Proteins
    B. Fats
    C. Carbohydrates
    D. Vitamins

Answer: B

30. Site for fatty acid biosynthesis within cell is __________.
    A. Mitochondria
    B. Ribosome
    C. Cytoplasm
    D. Chloroplast

Answer: C

Complete each sentence or statement.

1. The synthesis of ketone bodies from acetyl-CoA is called ________.

Answer: ketogenesis

2. A/An ________ is a protein that carries out more than one chemical reaction.

Answer: multifunctional enzyme

3. A cholesterol derivative that acts as a detergent to solubilize lipids for digestion and absorption is ________.

Answer: bile acid

4. ________ is a eukaryotic organelle with specialized oxidative functions, which include fatty acid degradation.

Answer: Peroxisome

5. A globular particle, containing lipids and proteins, that transports lipids between tissues via the bloodstream is a/an ________.

Answer: lipoprotein

6. ________ are compounds produced from acetyl-CoA by the liver and used as metabolic fuels in other tissues when glucose is unavailable.

Answer: Ketone bodies

7. A fatty acid that an animal cannot synthesize and must therefore obtain in its diet is called a/an ________.

Answer: essential fatty acid

8. ________ is a disease characterized by accumulation of lipids in the walls of blood vessels.

Answer: Atherosclerosis

9. Before they are oxidized, fatty acids are "activated" by attachment to ________.

Answer: coenzyme A or CoA

10. The usual product of fatty acid synthesis is a 16-carbon molecule called ________.

Answer: palmitate or palmitic acid

11. ________ are signaling molecules derived from the 20-carbon fatty acid arachidonate.

Answer: Eicosanoids or Prostaglandins

12. Drugs known as statins inhibit HMG-CoA reductase and thereby prevent the synthesis of ________.

Answer: cholesterol

13. Acetyl-CoA carboxylase converts the acetyl group of acetyl-CoA to a three-carbon ________ group.

Answer: malonyl

14. Methylmalonyl-CoA mutase, which catalyzes a molecular rearrangement reaction, is one of only a few enzymes with a ______ cofactor.

Answer: cobalamin or vitamin B12

15. A process of attachment of fatty acids to glycerol to form triglycerides is called ______________.

Answer: Esterficatrion

“This Page is Intentionally Left Blank”

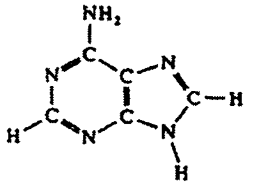
Chapter 11

Nucleic Acid Metabolism

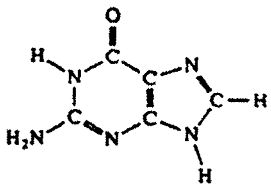
Purine Metabolism

The Bases

Purines:

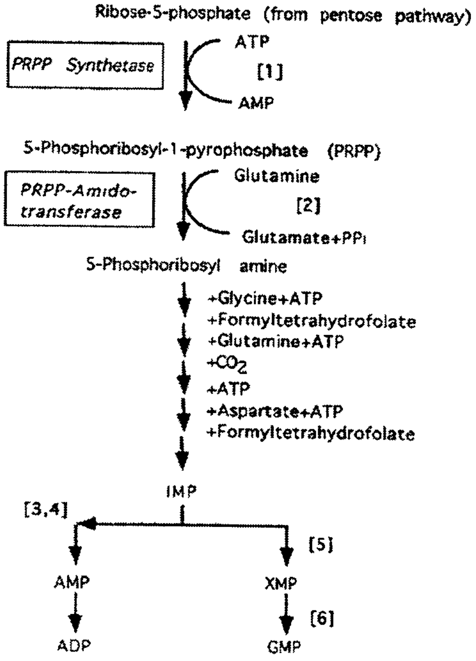


Adenine



Guanine

Purine Biosynthesis: (Fig. 11.1)



Ribose-5-phosphate (from pentose pathway)
PRPP Synthetase
ATP
[1]
AMP
5-Phosphoribosyl-1-pyrophosphate (PRPP)
PRPP-Amido-transferase
Glutamine
[2]
Glutamate+PPi
5-Phosphoribosyl amine
+Glycine+ATP
+Formyltetrahydrofolate
+Glutamine+ATP
+CO2
+ATP
+Aspartate+ATP
+Formyltetrahydrofolate
IMP
[3,4]
[5]
AMP
XMP
[6]
ADP
GMP

Fig. 11.1 : Schematic Presentation of Purine Biosynthesis

[1] 5-Phosphoribosyl-1-pyrophosphate (PRPP) synthesis is catalyzed by PRPP synthetase. Note: the ribose-5-phosphate for the pathway comes from the Pentose Phosphate Pathway (see "PPP/Gluconeogenesis" Lecture).

[2] The committed, regulated step in the pathway catalyzed by PRPP amidotransferase. Note: glutamine provides the nitrogen to initiate purine synthesis.

Inosinic acid (IMP) is the first purine formed, overall 6 high energy phosphate bonds are consumed to produce IMP.

[3] IMP + Aspartate + GTP ---> Adenylosuccinate; Enzyme: **Adenylosuccinate Synthetase.**

[4] Adenylosuccinate ---> AMP + Fumarate; Enzyme: **Adenylosuccinase.**

[5] IMP + NAD^+ ---> XMP + NADH + H^+; Enzyme: **IMP Dehydrogenase.**

[6] XMP + Glutamine + ATP ---> GMP + Glutamate + AMP + PP_i.

Regulation of Purine Biosynthesis

The PRPP amidotransferase enzyme exists as an active monomer and an inactive polymer (see "Introduction to Metabolism" Lecture). IMP, GMP and AMP all inactivate the enzyme causing a shift towards the polymerized inactive form. PRPP causes a shift towards the active monomeric form.

AMP is a competitive inhibitor (see "Enzymes:Catalysis and Kinetics" Lecture) of Adenylosuccinate Synthetase, GMP competitively inhibits IMP Dehydrogenase. Note: GTP is required for AMP synthesis and ATP is required for GMP synthesis, hence there is coordinated regulation of these nucleotides.

Purine Degradation (Fig. 11.2)

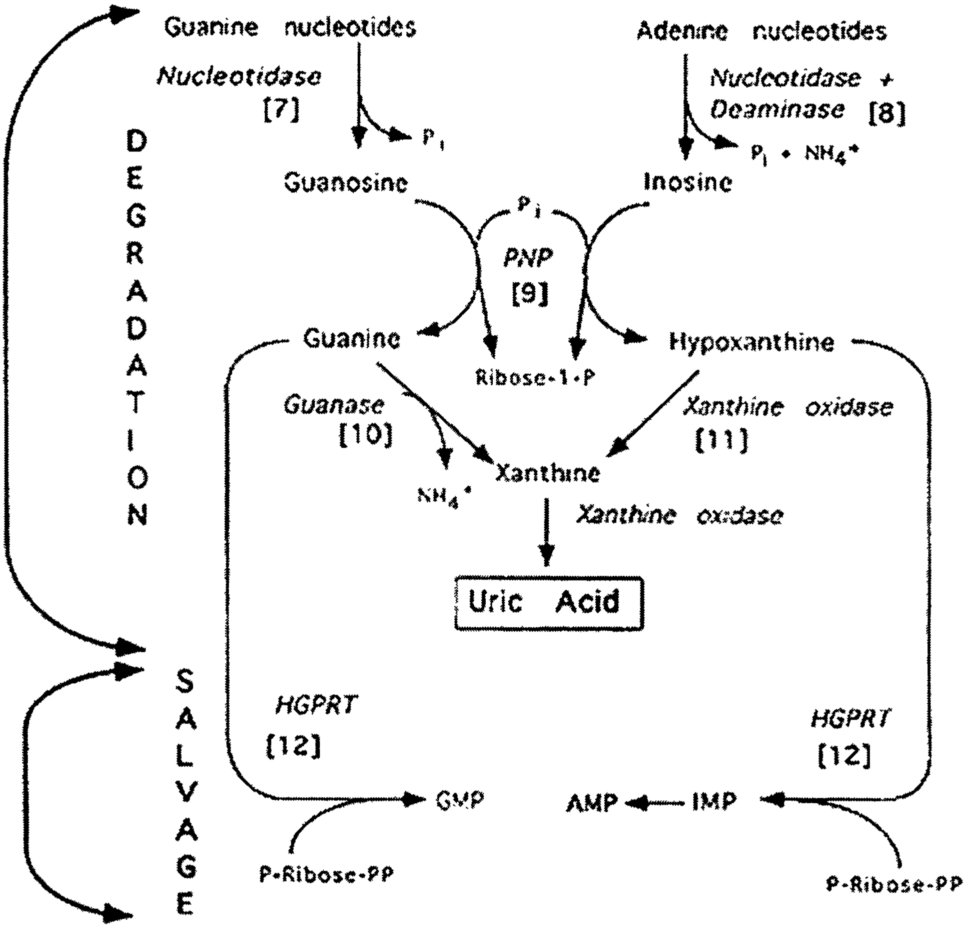

Guanine nucleotides
Adenine nucleotides
Nucleotidase
[7]
P_i
Nucleotidase +
Deaminase [8]
P_i + NH_4^+
Guanosine
Inosine
P_i
PNP
[9]
Guanine
Ribose-1-P
Hypoxanthine
Guanase
[10]
Xanthine oxidase
[11]
NH_4^+
Xanthine
Xanthine oxidase
Uric Acid
DEGRADATION
SALVAGE
HGPRT
[12]
HGPRT
[12]
GMP
AMP
IMP
P-Ribose-PP
P-Ribose-PP

Fig. 11.2 : Schematic Presentation of Purine Degradation

Note: **PNP** = Purine Nucleotide Phosphorylase; **HGPRT** = Hypoxanthine-Guanine Phosphoribosyl Transferase

[7] **Nucleotidase**: Purine Mononucleotide + H_2O ---> Purine Nucleoside + P_i.

[8] **Deaminase**: Adenosine + H2O ---> Inosine + NH_4^+.

[9] **Purine Nucleoside Phosphorylase**: Inosine + P_i ---> Hypoxanthine + R-1-P; Guanosine + P_i ---> Guanine + R-1-P; Note: R-1-P can be converted to R-5-P.

[10] **Guanase** deaminates guanine to xanthine.

[11] **Xanthine Oxidase** oxidizes hypoxanthine to xanthine, then xanthine to uric acid which is excreted in the urine. Co-factors: Mo, Fe^{3+}, FAD, O_2.

Note: H_2O_2, a product of this reaction must be scavenged by Glutathione Reduced (see "PPP/Gluconeogenesis" Lecture).

[12] Purines can be salvaged after steps [7] and [8] by reconverting the nucleosides to nucleotides via nucleotide kinases. The free base can also be saved in a salvage pathway consisting of a single enzyme, HGPRT: Hypoxanthine + PRPP ---> IMP + PP_i; Guanine + PRPP ---> GMP + PP_i . The importance of this pathway as a source of nucleotides is shown by the consequences of a deficiency of HGPRT known as Lesch-Nyhan syndrome. This pathway reduces the need and energy expenditure of biosynthesis by maintaining nucleotide levels.

Clinical Correlates

Gout: is associated with either increased formation of uric acid or its decreased renal excretion. Its incidence is relatively high, occurring in about 0.3% of the population. Gout produces a painful arthritis, particularly in the joints of the extremities. There are two broad types of gout: primary and secondary. Primary gout, of which there are several types, is inherited. Secondary gout is brought on by a variety of disorders such as leukaemia.

Individuals with a glucose-6-phosphatase (G-6-P ---> Glucose, in the liver) deficiency develop a glycogen storage disease. This condition results in hypoglycemia, low blood sugar, accumulation of lactic acid and ketones. Distal kidney tubule excretion of uric acid is inhibited and hyperuricemia (XS uric acid) and gout results.

Treatment: the drug that most effectively inhibits the formation of uric acid is allopurinol, a competitive inhibitor of xanthine oxidase. Hypoxanthine and xanthine are excreted during allopurinol therapy. Allopurinol, as with guanine and hypoxanthine, can be converted to its ribonucleotide form by HGPRT. Reducing the formation of uric acid with allopurinol relieves the symptoms of gout and decreases the possibility that uric acid kidney stones will form.

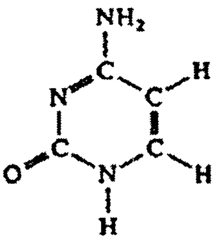
Lesch-Nyhan Syndrome: produces a tremendous overproduction of uric acid. The defect in Lesch-Nyhan disease is the gene for the enzyme HGPRT. The lack of HGPRT activity is virtually complete and the disease is X-linked recessive and thus limited to males. It has a very early age of on-set and is characterized by extremely aggressive behaviour that generally leads to self-mutilation. Initially

HGPRT was considered a minor player in the "salvage" pathway that permitted reutilization of purine bases. The severity of Lesch-Nyhan syndrome suggests a more important role. Most likely the enzyme has an essential role in non-hepatic tissues where biosynthesis of purines occurs at a very low rate. Non-hepatic tissues contain the enzyme but depend on circulating purines and nucleosides to form nucleotides.

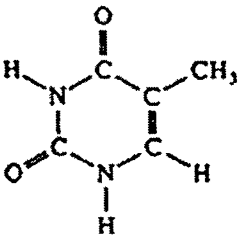
Pyrimidine Metabolism

The Bases

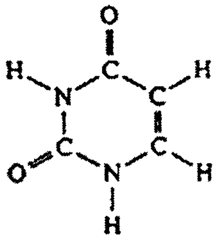
Pyrimidines:



Cytosine



Thymine



Uracil

Pyrimidine Biosynthesis (Fig. 11.3)

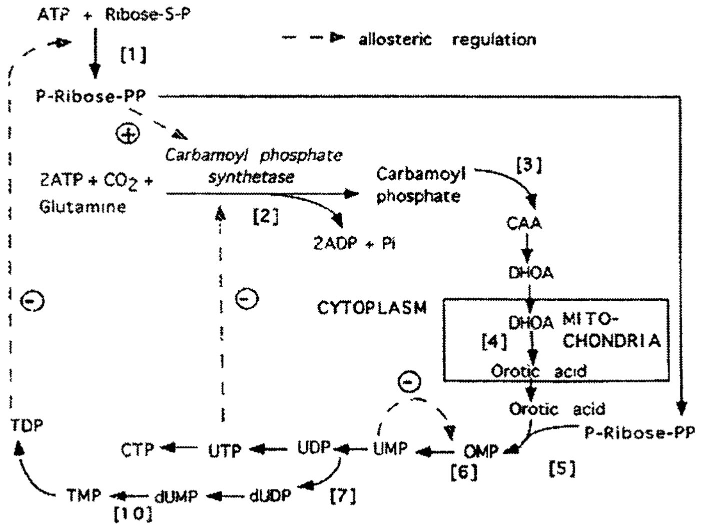

ATP + Ribose-5-P
[1]
allosteric regulation
P-Ribose-PP
⊕
Carbamoyl phosphate synthetase
2ATP + CO2 + Glutamine
[2]
2ADP + Pi
Carbamoyl phosphate
[3]
CAA
DHOA
⊖
⊖
CYTOPLASM
DHOA
MITO-CHONDRIA
[4]
Orotic acid
⊖
Orotic acid
P-Ribose-PP
[5]
OMP
[6]
UMP
UDP
UTP
CTP
[7]
dUDP
dUMP
TMP
[10]
TDP

Fig. 11.3 : Schematic Presentation of Pyrimidine Biosynthesis

Note: CAA = Carbamoyl Aspartate; DHOA = Dihydroorotate; OMP = Orotidine Monophosphate

[1] **PRPP Synthetase** (see "Purine Lecture")

[2] **Carbamoyl Phosphate Synthetase II** (glutamine):

Glutamine + CO_2 + 2 ATP ----> Carbamoyl phosphate + Glutamate + 2 ADP + P_i.

Carbamoyl Phosphate Synthetase II (CPS-II) differs in several ways from its isoform (CPS-I), the enzyme which provides carbamoyl phosphate for the Urea cycle (see "Protein Turnover / Ammonia Metabolism").

1. CPS-II is located in the cytoplasm, CPS-I mitochondria.
2. Glutamine not Ammonia is the Nitrogen source.
3. CPS-II does not require N-acetylglutamate as an allosteric activator
4. CPS-I is found only in Liver and Kidney, CPS-II in most tissues

[3] **Aspartate Carbamoyl Transferase**: the committed step Carbamoyl phosphate + Aspartate ----> Carbamoyl aspartate + P_i

[4] **Dihdroorotate Dehydrogenase**: the only Mitochondrial enzyme in the pathway Dihydroorotate + NAD^+ ----> Orotate + NADH + H^+

[5] **Orotate Phosphoribosyltransferase**: Orotate + PRPP ----> OMP + PP_i

[6] **OMP Decarboxylase**: OMP ----> UMP + CO_2

OMP is the first pyrimidine formed and is immediately decarboxylated to produce UMP. Nucleotides are then formed subsequently from UTP via CTP Synthetase.

Regulation of Pyrimidine Synthesis

The primary site of regulation is Carbamoyl Phosphate Synthetase II (glutamine) which is allosterically inhibited by UTP. Elevated PRPP increases the CPS-II activity to help control PRPP levels. Feedback inhibition (control) is provided by TDP inhibition of PRPP synthesis and UMP inhibition of OMP Decarboxylase.

Deoxyribonucleotide Synthesis

[7] Synthesis of Thymidine nucleotides first requires deoxyribonucleotide synthesis. The enzyme responsible for this step is Ribonucleotide Reductase. This enzyme acts on oxynucleotides in their diphosphate form. Thioredoxin, a small protein, is oxidized as the 2' hydroxyl group on the ribose ring is reduced. Oxidized Thioredoxin (S-S) is then reduced by $FADH_2$ and NADPH. The products are the respective deoxynucleotide diphosphates which are further phosphorylated and then used for DNA synthesis.

The exception dUDP is first converted to thymidine nucleotide via dUMP.

[8] A deficiency of Adenosine Deaminase (ADA) decreases metabolism of deoxyadenosine causing dATP to accumulate.

[9] Similarly a deficiency of Purine Nucleoside Phosphorylase causes accumulation of dGTP. In both cases Ribonucleotide Reductase is inhibited by accumulation of these nucleotides.

[10] Synthesis of thymidylate requires uridine nucleotide to be converted to the deoxy form. The substrate for Thymidylate Synthetase is dUMP, the N^5,N^{10}- methylene-tetrahydrofolate (CH_3-THF) is the "methyl" donor. This form of folate derives its carbon from serine:

Serine + THF ----> Glycine + CH_3-THF

Pyrimidine Salvage and Metabolism

Salvage Pathways:

Pyrimidine Phosphoribosyl Transferase: Pyrimidine base + PRPP ----> Nucleoside monophosphate + PP_i

Nucleoside Kinase: Nucleoside + ATP ----> Nucleotide + ADP

Metabolism: Uracil is degraded to β-alanine and Thymine to β-aminoisobutyrate.

Clinical Correlate: ***Chemotherapy***

5-Fluorouracil: is a pyrimidine analog which is converted metabolically to its toxic form, fluorodeoxyuridylate (F-dUMP). As cells metabolically activate the drug it acts as a "suicide" inhibitor of Thymidylate Synthetase.

Methotrexate (MTX, amethopterin): is a folic acid analog used to inhibit Dihydrofolate Reductase.

Inhibition of the Reductase affects folate metabolism leading to decreased glycine formation from serine and decreased purine synthesis which requires CH_3-THF. To facilitate normal cells folinic acid (Leucovorin) is given along with methotrexate. This acid aids normal cells by its conversion to the coenzyme of Thymidylate Synthetase, thus bypassing the block. Since the thymidine nucleotide requirements of rapidly proliferating cells are much greater than for quiescent cells folinic acid cannot meet the demands of the cancer cells.

Deoxycoformycin inhibits Adenosine Deaminase [8] to increase the levels of dATP which inhibits Ribonucleotide Reductase. Infusing Adenosine Arabinoside together with Deoxycoformycin increases inhibition of the Reductase further via elevated levels of both dATP and arabinoside-ATP. Since deoxynucleotides are essential for DNA synthesis this strategy is effective in treating certain types of leukemia.

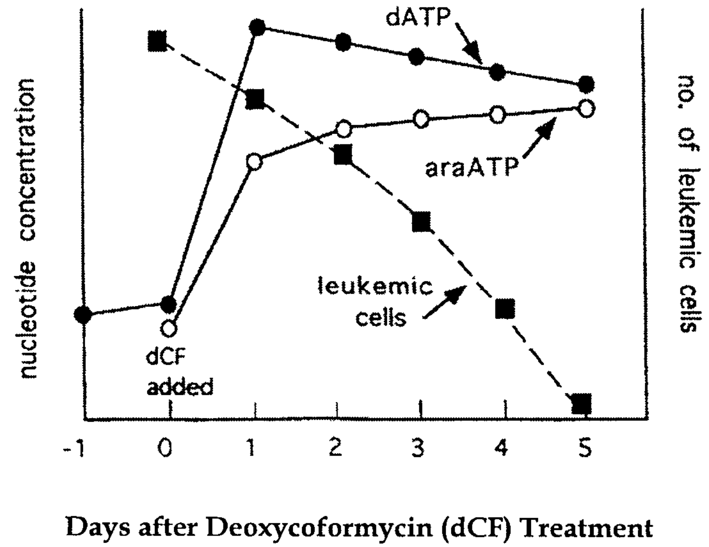


Fig. 11.4 : Relationship between Deoxycoformycin and Leukemia cells.

RNA Synthesis

There are a number of different types of RNA, which play different roles in the cell:

- mRNA - encodes proteins.
- rRNA - forms the ribosome, including the active site for peptide bond formation.
- tRNA - adaptor, binds amino acids and rRNA and translates between mRNA and protein.
- snRNA - small nuclear RNA, forms snRNPs, which process mRNA by removing introns.
- snoRNA - small nucleolar RNA, forms snoRNPs, which process rRNA, mostly by methylation and isomerisation.
- siRNA - small interfering RNA, involved in gene silencing and regulation.
- gRNA - guide RNA, needed for RNA editing, the removal and insertion of bases into mRNA.

- tmRNA - an RNA molecule that disengages ribosomes from stalled translation of mRNA in bacteria.
- telomerase RNA - an RNA molecule that forms much of the structure and all of the template required by telomerase.
- hnRNA - rag-bag of unprocessed pre-mRNA transcripts and other heterogeneous nuclear RNAs of less well defined function.

The process of synthesizing RNA from the genetic information encoded by DNA is called transcription. The enzymes involved in transcription are called RNA polymerases. Prokaryotes have one type; eukaryotes have three types of nuclear RNA polymerases.

RNA polymerase is the enzyme that generates RNA from DNA. Cells contain 20 times more RNA than DNA: in fact, about 5% of the cell is RNA, although only 5% of this 5% is mRNA, because most of the RNA in the cell is rRNA. Since the majority of RNA is rRNA, significantly more RNA is transcribed than translated. This is especially true in eukaryotes, whose mRNA requires processing to remove introns.

The discovery of RNA polymerase is an instructive one. In 1955, Grunberg-Manago and Ochoa found an enzyme catalysing:

$$(RNA)_n + NDP \longrightarrow (RNA)_{n+1} + P_1.$$

The primary gene products of RNA polymerase (in eukaryotes) are:

- (pre-)mRNA (messenger);
- rRNA (ribosomal);
- tRNA (transfer);
- snRNA (small nuclear - spliceosomes);
- Other hnRNA (heterogeneous nuclear, such as snoRNA - small nucleolar).

The interactions of RNA after (and indeed before) transcription is now understood to be absolutely fundamental to the functioning of the cell: RNA is no longer considered to be a bland intermediate between DNA and protein, but rather an active participant in its own synthesis, processing and regulation.

RNA polymerase enzymes are very large:

- 100 kDa in T7 bacteriophage.
- 400 kDa in bacteria.
- 500 kDa for eukaryotes.

Their speed of transcription is around 50 b/s, meaning an mRNA for an average protein takes about 20 s (prokaryote) or 3 min (eukaryote) to transcribe - longer in eukaryotes, despite their proteins being of similar size, because they contain introns.

The prokaryotic RNA polymerase consists of a core enzyme and an auxiliary protein factor called sigma. (σ factor). The core consists of four subunits, two are identical, α, the other two similar, β and β'. The β' subunit binds the DNA while the β subunit binds the nucleotides that are to be joined together to form the RNA molecule. Sigma factors function in identifying specific DNA sequences known as promoters. Promoters are sites that tell the RNA polymerase where to begin transcription.

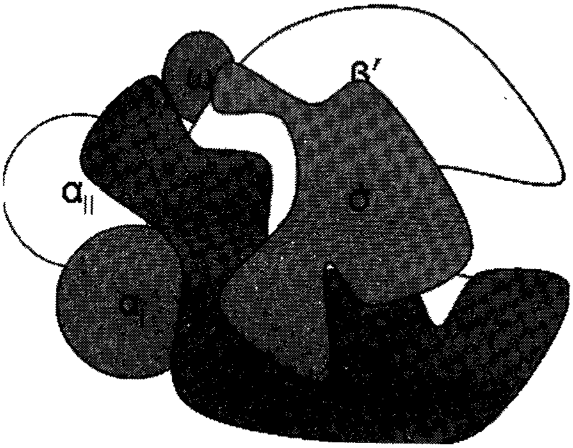


Fig. 11.5 : Prokaryotic RNA Polymerase

All multi-subunit RNA polymerases have 5 core subunits. The (true) Bacteria have an additional σ factor subunit that aids regulation and binding to DNA.

Eukaryotes have four different RNA polymerases (RNA pol). Three are required for transcription of nuclear genes and the fourth for transcription of mitochondrial genes (Fig. 11.6). RNA polymerase I transcribes ribosomal RNA (rRNA), pol II transcribes mRNA and pol III tRNA and several small RNA's. The three polymerases consist of ten or more subunits. All have two large subunits with homology to the β and β' subunits of the prokaryotic RNA polymerase. The three eukaryotic polymerases can be distinguished based on their sensitivity to α-amanitin, a toxin found in some types of mushrooms. RNA pol II activity is severely inhibited, pol III weakly and pol I is insensitive. The antibiotic rifampicin inhibits prokaryotic RNA polymerases.

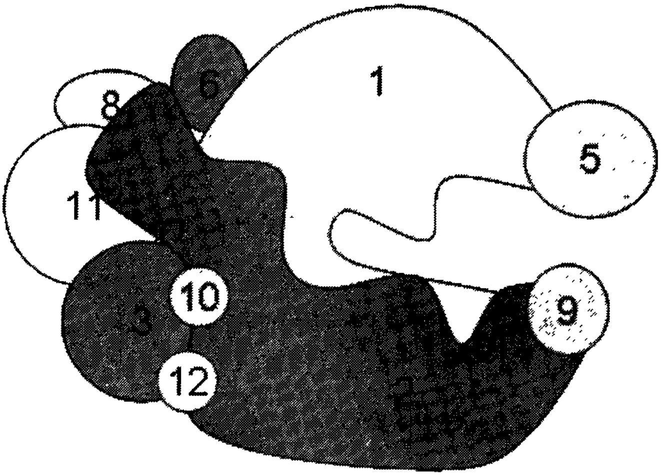


Fig. 11.6 : Eukaryotic RNA Polymerase

Eukaryotic RNA polymerases have five core subunits plus five common subunits.

Eukaryotes have three varieties of RNA polymerase. These differ slightly from one another: RNA polymerase II has the five core subunits plus the five common subunits, but also has two 'variable' subunits specific to RNA polymerase II only. Note that RNA polymerase II has a 'tail', which is involved in RNA processing and the initiation of transcription (Fig. 11.7).

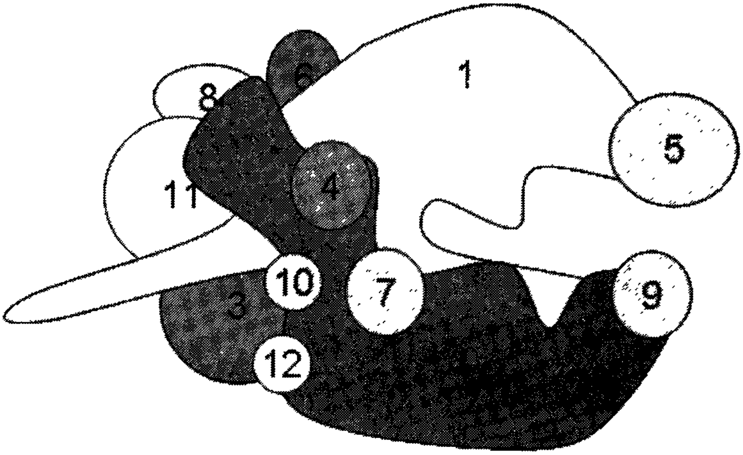


Fig. 11. 7 : Eukeryotic RNA Polymerase - II

RNA polymerase cycle of prokaryotes (Fig. 11.8)

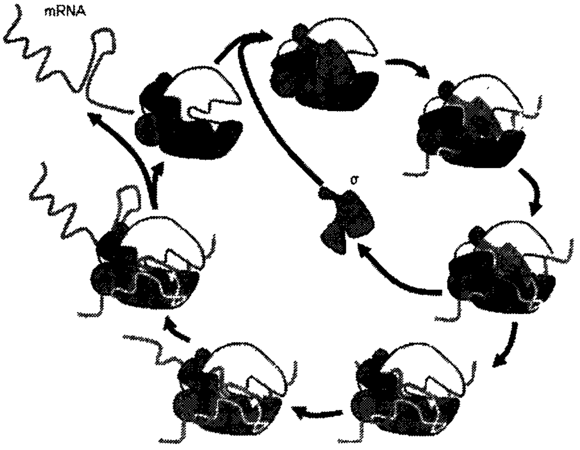


Fig. 11.8 : Schematic presentation of mRNA synthesis in Prokaryotes

Eukaryotic RNA Polymerases

Eukaryotes have three RNA polymerases which synthesized different type of RNA

- RNA polymerase I - rRNA.
- RNA polymerase II - mRNA.
- RNA polymerase III - tRNA.

All Three have the core Subunits: $\alpha_I\alpha_{II}$ are 3,11 in eukaryotes (heterodimer), become 2,1 and ω's equivalent is 6. All three also have five common extra subunits: 5, 8, 9, 10 and 12, and a variable number of specific subunits.

Transcription in eukaryotes differs markedly from that in prokaryotes (bacteria).

- Eukaryote polymerase is larger, with more subunits.
- The equivalent of the 'α' subunits are not identical.
- RNA polymerase cannot bind DNA directly: it requires transcription factors.
- Nucleosomes must be dealt with.
- It requires helicase (bacterial enzyme has inherent helicase activity).

There are three phases of transcription: initiation, elongation and termination.

Initiation

The initiation of transcription is directed by DNA sequences called promoters which tell the RNA polymerase where to begin transcription. The subunits that enable RNA polymerases to recognize and bind promoters are called initiation factors. The initiating nucleotide can be either a purine or pyrimidine. There are numerous eukaryotic promoters with multiple promoter sequence elements. Some of the elements specify where transcription is to be initiated, others determine the frequency with which transcription is initiated at a specific gene. The initiation of transcription in eukaryotes is complicated and involves numerous factors (proteins) that must interact with the DNA and with one another to initiate transcription.

Promoters

1. Only one strand of the DNA that encodes a promoter, a regulatory sequence, or a gene needs to be written.
2. The strand that is written is the one that is identical to the RNA transcript, thus the antisense strand of the DNA is always selected for presentation.
3. The first base on the DNA where transcription actually starts is labelled +1.
4. Sequences that precede, are upstream of the first base of the transcript, are labeled with negative numbers. Sequences that follow the first base of the transcript, are downstream, are labeled with positive numbers.

RNA pol II promoters are quite diverse. This enables the cell to choose and regulate the expression of the 50 to 100 thousand different genes encoded by its DNA. There are some sequence elements that are conserved and found in most RNA pol II promoters. There are three "boxes": TATA usually found 25 to 35 base pairs upstream, the CAAT box and the GC box both located from 40 to 200 base pairs upstream (Fig 11.9).

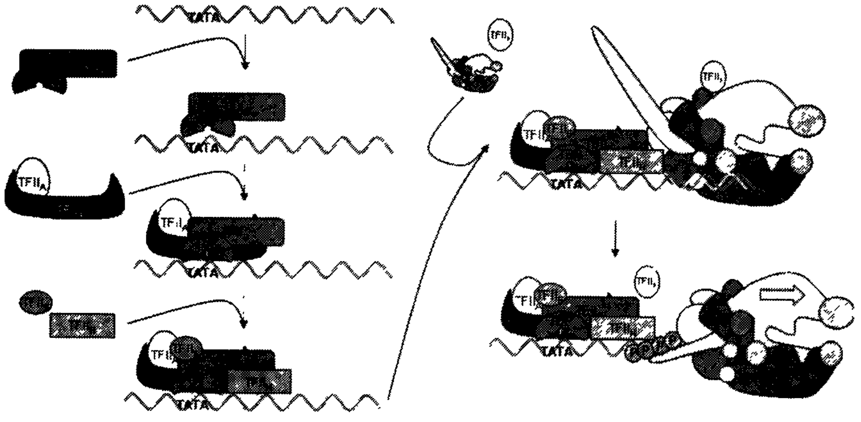


Fig. 11.9 : RNA Pol. II Promoters

Initiation in RNA polymerase II

These three elements provide a basal level of transcription and are found in most "housekeeping" genes. Housekeeping genes encode enzymes and proteins that all cell types require for normal function and are usually expressed at steady state or basal levels. Other sequence elements, which are continually being discovered, serve as regulatory elements. Elements that enable a cell to specifically turn other non-housekeeping genes on or off in response to environmental signals such as hormones, growth factors, metals and toxins. The spacing and orientation of all of the sequence elements are critical for proper functioning. There is a third type of sequence element that can be located either upstream or downstream relative to the initiation site which is called an enhancer or silencer. Enhancers or silencers affect the rate and frequency of initiation of transcription.

RNA pol III promoters for tRNA are found downstream of the initiation point. These promoters consist of two elements, the first of which is located 8 to 30 base pairs downstream and is called Box A. The second element is 50 to 70 base pairs downstream and is called Box B.

RNA pol I promoter consists of a 70 base pair long core element and an upstream element that is about 100 base pairs long. The core spans a segment of DNA that includes sequences that are both up and downstream of the initiation site.

Elongation

RNA polymerase links ribonucleotides together in a 5' to 3' direction. The polymerase induces the 3' hydroxyl group of the nucleotide at the 3' end of the growing RNA chain which attacks (nucleophilic) a phosphorous of the incoming ribonucleotide. A diphosphate is released and the 5' carbon of the incoming nucleotide is linked through a phosphodiester bond to the 3' carbon of the preceding nucleotide (Fig 11.10).

Nucleotide incorporation is determined by base pairing with the template strand of the DNA. The template is the DNA strand, also called the sense strand, that is copied by the RNA polymerase into a complementary strand of RNA called the transcript. The DNA strand that is not copied is know as the antisense strand. Note that while the RNA chain grows in a 5' to 3' direction the polymerase migrates along the sense strand in a 3' to 5' direction. Thus, the 5' to 3' ribonucleotide sequence of the RNA transcript is identical to the 5' to 3' antisense DNA strand with uracil in place of thymidine.

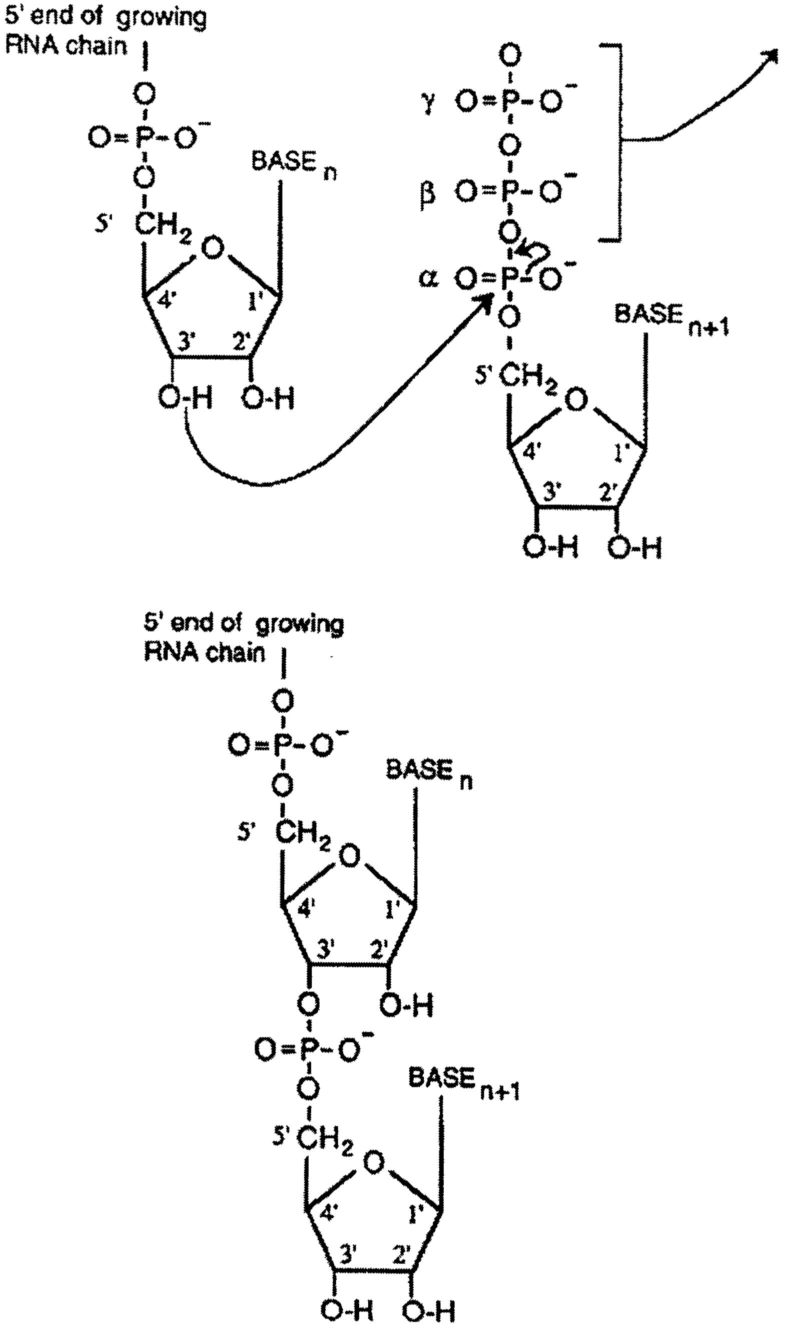

5' end of growing RNA chain
O=P-O⁻
5' CH₂
BASE n
4'
1'
3'
2'
O-H
O-H
γ O=P-O⁻
β O=P-O⁻
α O=P-O⁻
BASE n+1
5' CH₂
5' end of growing RNA chain
BASE n
BASE n+1

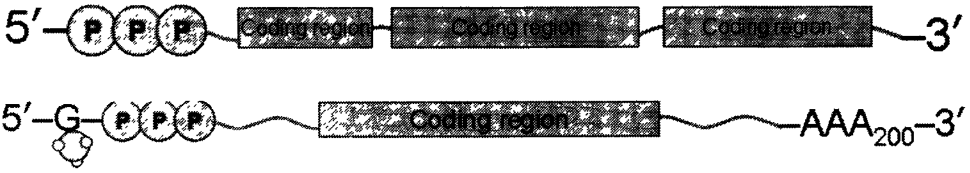
Fig. 11.10 : RNA Elongation by RNA Polymerase

Termination

Prokaryotes use two means for terminating transcription, factor-independent and factor-dependent. Certain DNA sequences function as signals that tell the RNA polymerase to terminate transcription. The DNA of a terminator sequence encoded an inverted repeat and an adjacent stretch of uracils. Factor-dependent termination involves a terminator sequence as well as a factor or protein called rho. The mechanisms by which eukaryotes terminate transcription are poorly understood. Most eukaryotic genes are transcribed for upto several thousand base pairs beyond the actual end of the gene. The excess RNA is then cleaved from the transcript when the RNA is processed into its mature form.

RNA Processing

Most transcripts must be processed before becoming fully functional. Most eukaryotic RNA must be transported across the nuclear membrane where it is processed then transported to the cytosol. Processing helps stabilize and protect the RNA so it can function in the cytosol and also functions in regulating the expression of certain genes.



Mature **mRNA** is formed by extensively modifying the primary transcript also called heterogeneous nuclear RNA (hnRNA). The hnRNA must undergo three major modifications before maturing into mRNA: capping, polyadenylation and splicing.

The roles of this modification are several:

- Prevent degradation by exonucleases: cells contain RNAses (anti-viral).
- Specific functions (*e.g.,* binding to ribosomes, removal of non-coding sequences, alternative splicing).

Capping: all mRNA's are capped at their 5' ends with 7-methylguanylate. Guanylyl transferase catalyzes the linking of 7'-methylguanylate to the mRNA through a 5' to 5' triphosphate bridge. The capping positions the mRNA onto the 40S preinitiation complex and protects it from exonuclease activity (Fig 11.11).

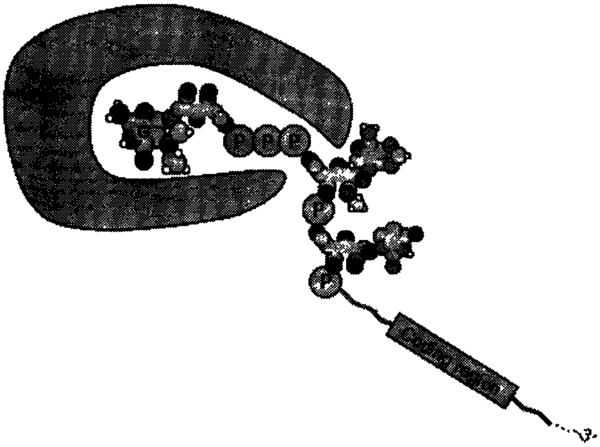

P
P
P
P
P
Coding region
3'

Fig 11.11 : mRNA Capping

Polyadenylation: is the addition of a chain of adenylate residues, known as a poly A tail to the 3' terminus of mRNA (Fig 11.12). After the RNA is cut, an enzyme poly A polymerase, catalyzes the polymerization of adenylates. The poly A tail slows the exonucleolytic degradation of mRNA, once the tail is removed mRNA is quickly degraded.

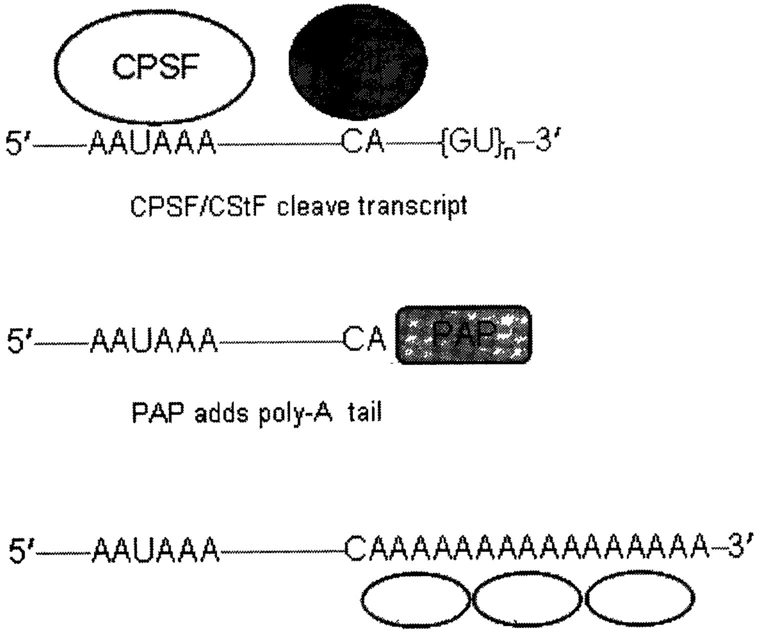
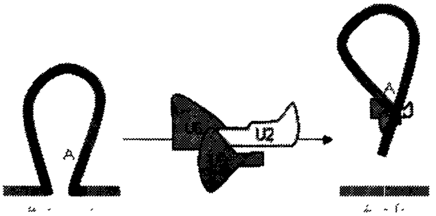
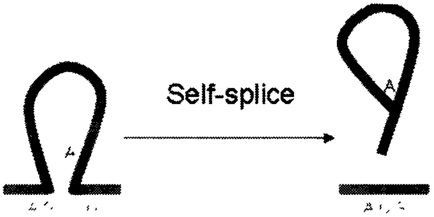

CPSF
5'—AAUAAA——CA—{GU}n–3'
CPSF/CStF cleave transcript
5'—AAUAAA——CA
PAP
PAP adds poly-A tail
5'—AAUAAA——CAAAAAAAAAAAAAAA–3'

Fig 11.12 : Poly-A tail bound by PABP

Splicing: is the removal of noncoding sequences, derived from the DNA template, from the hnRNA to form a functional mRNA. The noncoding sequences are called introns while the coding sequences are known as exons. All introns have the sequence GU at their 5' ends and AG at their 3' ends. The guanyl residue at the 5' end of the intron is linked by a 2' to 5' phosphodiester linkage to an adenylate residue within the intron. The result is a lariat (loop) structure and the release of the 3' end of the first exon. The 3' end of the intron is spliced by an enzyme known as a spliceosome, which releases the loop and frees the 5' end of the second exon. The exons are then joined together.

5′---*E1*---**I1**---*E2*---**I2**---*E3*---**I3**---*E4*---**I4**---*E5*---3′



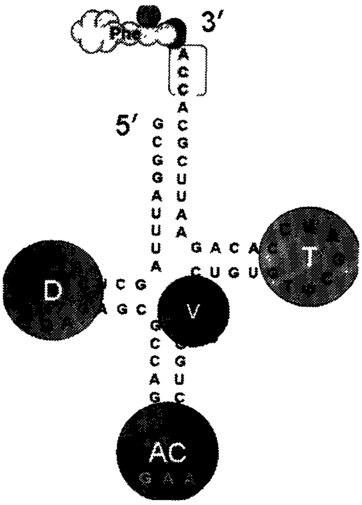


The **rRNA** of both prokaryotes and eukaryotes are synthesized as large precursors. The precursor rRNA's are processed into their mature form by nucleases and methylases.

The **tRNA's** of both prokaryotes and eukaryotes are also transcribed as precursors which are cleaved and extensively modified.

tRNA has 'L'-shaped tertiary structure.

- D(ihydrouridine) loop.
- T(hymine) loop.
- Anticodon loop.
- Acceptor stem.
- Variable loop.



DNA Synthesis

- DNA replication occurs during S-phase and is initiated at origins of replication. It is semiconservative in nature.
- Replication occurs at replication forks: two of these moving out from an origin form a 'bubble'.
- DNA is only synthesised in the 5′ to 3′ direction: the leading strand is synthesised continuously, the lagging strand in discontinuous Okazaki fragments. This allows proofreading.
- Replication requires coordination between helicase, primase, SSBPs, two polymerases, clamps, loaders, topoisomerases, ligases and RNAses.

The discovery of the double-helical nature of DNA by Watson & Crick explained how genetic information could by duplicated and passed on to succeeding generations. The strands of the double helix can separate and serve as templates for the synthesis of daughter strands. In conservative replication the two daughter strands would go to one daughter cell and the two parental strands would go to the other daughter cell. In semiconservative replication one parental and one daughter strand would go to each of the daughter cells.

Through experimentation it was determined that DNA replicates via a semiconservative mechanism. There are three possible mechanisms that can explain DNA's semiconservative replication.

DNA synthesis starts at a specific place on a chromosome called an origin. In the first mechanism one daughter strand is initiated at an origin on one parental strand and the second is initiated at another origin on the opposite parental strand. Thus, only one strand grows from each origin. Some viruses use this type of mechanism.

In the second mechanism replication of both strands is initiated at one origin. The site at which the two strands are replicated is called the replication fork. Since the fork moves in one direction from the origin this type of replication is called unidirectional. Some types of bacteria use this type of mechanism.

In the third mechanism two replication forks are initiated at the origin and as synthesis proceeds the two forks migrate away from one another. This type of replication is called bi-directional. Most organisms, including mammals, use bi-directional replication (Fig 11.13).

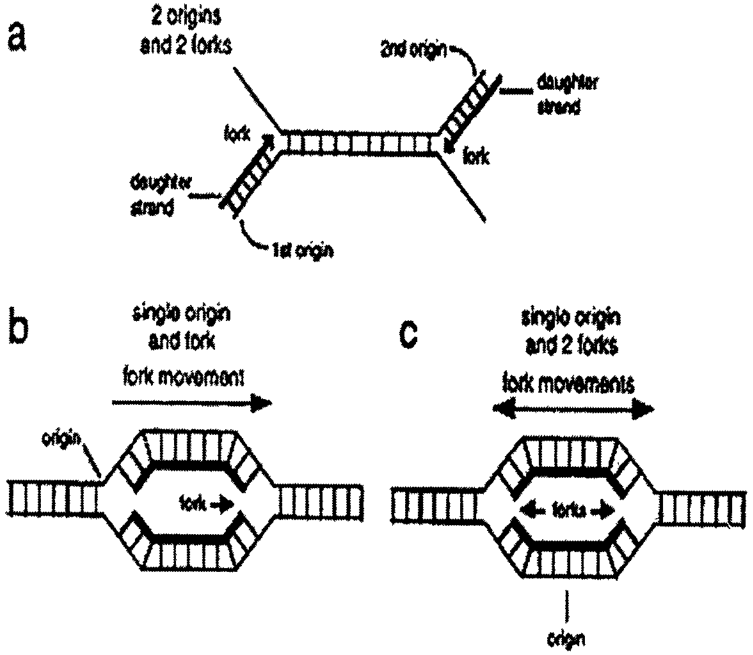

a
2 origins and 2 forks
2nd origin
daughter strand
fork
fork
daughter strand
1st origin
b
single origin and fork
fork movement
origin
fork
c
single origin and 2 forks
fork movements
forks
origin

Fig. 11.13 : Various DNA Replication Forks in Eukaryotic Chromosomes

However, Prokaryotic chromosomes only have one origin of replication (Fig. 11.14)

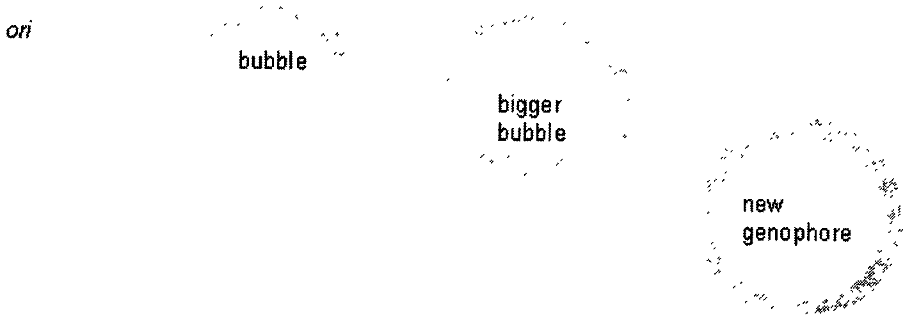

ori
bubble
bigger bubble
new genophore

Fig 11.14 : Single bubble for DNA Replication in Prokaryotic Chromosomes

Requirements for DNA Synthesis

There are four basic components required to initiate and propagate DNA synthesis. They are: substrates, template, primer and enzymes.

Substrates

Four deoxyribonucleotide triphosphates (dNTP's) are required for DNA synthesis (note the only difference between deoxyribonucleotides and ribonucleotides is the absence of an OH group at position 2' on the ribose ring). These are dATP, dGTP, dTTP and dCTP. The high energy phosphate bond between the α and β phosphates is cleaved and the deoxynucleotide monophosphate is incorporated into the new DNA strand.

Ribonucleoside triphosphates (NTP's) are also required to initiate and sustain DNA synthesis. NTP's are used in the synthesis of RNA primers and ATP is used as an energy source for some of the enzymes needed to initiate and sustain DNA synthesis at the replicating fork.

Template

The nucleotide that is to be incorporated into the growing DNA chain is selected by base pairing with the template strand of the DNA. The template is the DNA strand that is copied into a complementary strand of DNA.

Primer

The enzyme the synthesizes DNA, DNA polymerase, can only add nucleotides to an already existing strand or primer of DNA or RNA that is base paired with the template.

Enzymes

An enzyme, DNA polymerase, is required for the covalent joining of the incoming nucleotide to the primer. To actually initiate and sustain DNA replication requires many other proteins and enzymes which assemble into a large complex called a replisome. It is thought that the DNA is spooled through the replisome and replicated as it passes through.

DNA Polymerases

There are many types of DNA polymerases which can excise, fill gaps, proofread, repair and replicate.

Prokaryotes:

- DNA polymerase I - repair.
- DNA polymerase II - cleans up Okazaki fragments.
- DNA polymerase III - main polymerase.

Eukaryotes:

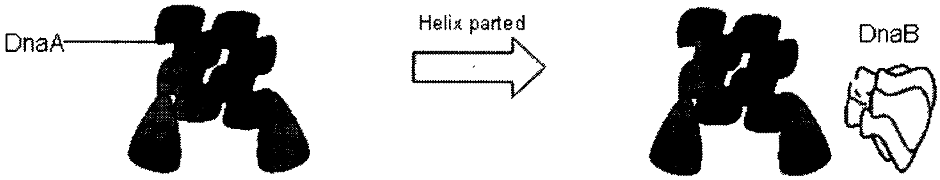
- DNA polymerase α - lagging strand priming.
- DNA polymerase β - repair.

- DNA polymerase γ - mitochondrial enzyme.
- DNA polymerase δ - leading strand and lagging strand elongation.
- DNA polymerase ε - leading strand (depends on species).

Other Factors Required for DNA Synthesis

Origins

Origins are unique DNA sequences that are recognized by a protein that builds the replisome. Origins have been found in bacterial, plasmid, viral, yeast and mitochondrial DNA but not in mammalian DNA. However since specific origins have been found in yeast chromosomes it is likely that specific origins are also used for initiating DNA replication in humans. Most origins have a site that is recognized and bound by an origin-binding protein. When the cis-acting factor binds to the origin the A + T rich sequence becomes partially denatured allowing other replication factors known as cis-acting factors to bind and initiate DNA replication.



Prokaryotic origins are initiated by DnaA, which loads in the DnaB helicase.

Trans-acting Factors

Origin-binding Protein: binds and partially denatures the origin DNA while binding to another enzyme called helicase.

Helicases: unwind double stranded DNA (Fig. 11.15).

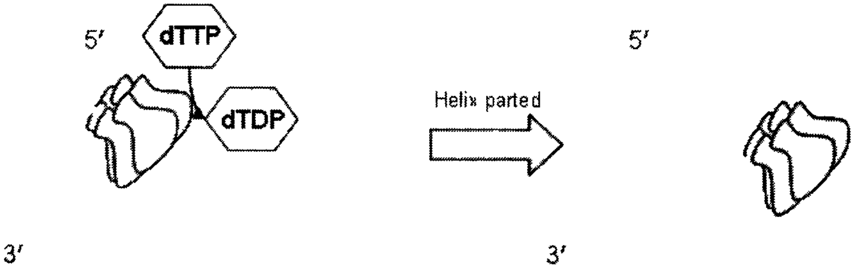


Fig. 11. 15 : DNA helicase uses the energy of dTTP to screw along a dsDNA molecule, melting the helix and parting the strands.

Single-stranded DNA Binding Protein (SSB): enhances the activity of the helicase and prevents the unwound DNA from renaturing (Fig 11.16).

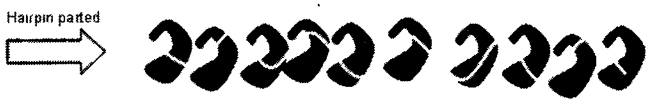


Fig 11.16 : SSBPs stabilise ssDNA and prevent it from re-annealing inappropriately.

Primase: synthesize the RNA primers required for initiating leading and lagging strand synthesis.

DNA Polymerase: recognizes the RNA primers and extends them in the 5' to 3' direction.

Processivity Factors: help load the polymerase onto the primer-template while anchoring the polymerase to the DNA.

Topoisomerase: removes the positive supercoils that form as the fork is unwound by the helicase. (Fig 11.17)

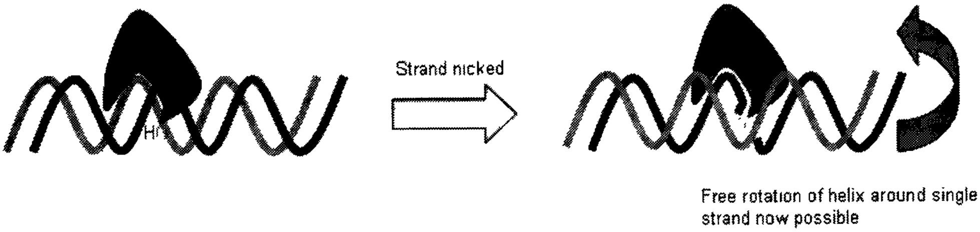


Fig. 11.17 : Topoisomerase I Allows Strain Relief Ahead of the Replication Fork.

Prokaryotic chromosomes are Möbius strips. Topoisomerase II creates gates by nicking both strands of the dsDNA, allowing helices to cross one another (Fig 11.18). Tangles and linked loops can therefore be separated. It also forms part of the eukaryotic nuclear scaffold, for similar reasons.

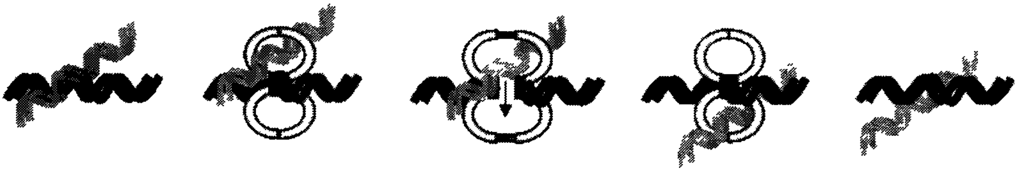


Fig. 11.18 : Topoisomerase II Allows DNA Molecules to Pass Through One Another.

RNaseH: removes RNA portions from Okazaki fragments.

Ligase: seals the nicks after filling in the gaps left by DNA polymerase.

DNA SYNTHESIS, 5' TO 3' (Fig. 11.19)

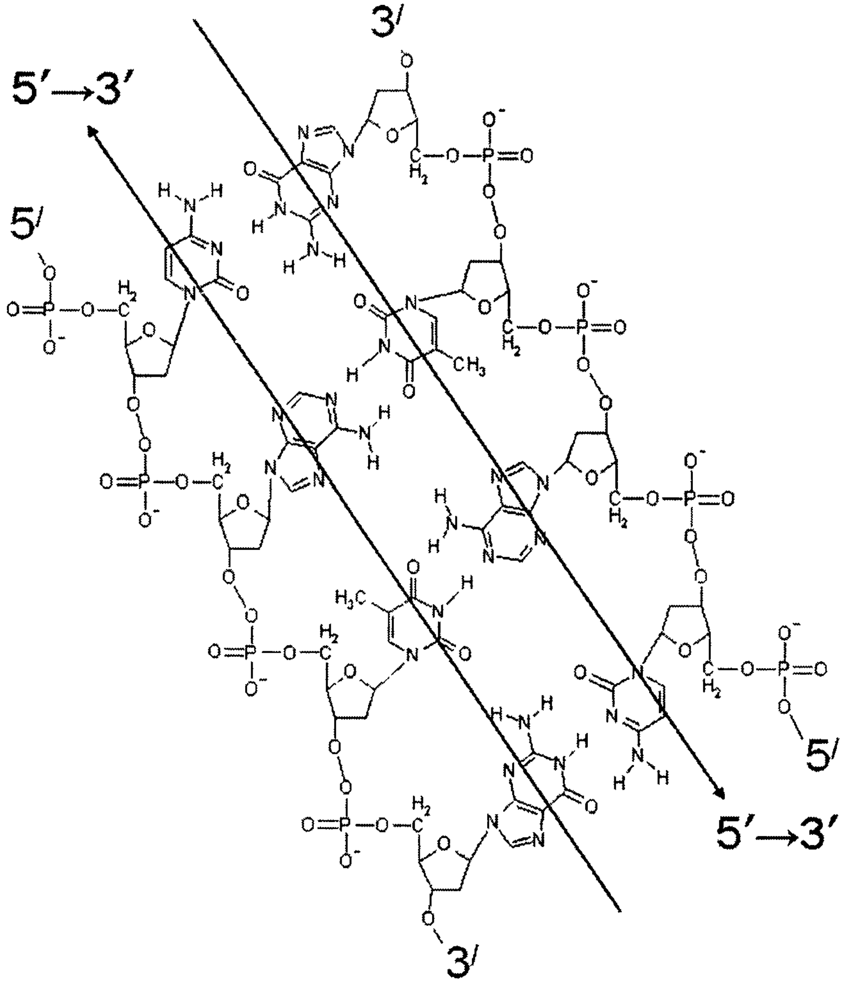
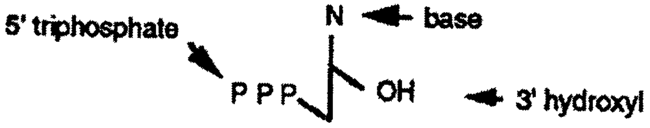


Fig. 11.19 : DNA Synthesis

DNA synthesis only occurs in the 5′ and 3′, so DNA polymerases must move in antiparallel directions to synthesise the two daughter helices.


5' triphosphate
N
base
P P P
OH
3' hydroxyl

The major catalytic step of DNA synthesis is shown below. Notice that DNA synthesis always occurs in a 5' to 3' direction and that the incoming nucleotide first base pairs with the template and is then linked to the nucleotide on the primer. (Fig 11.20)

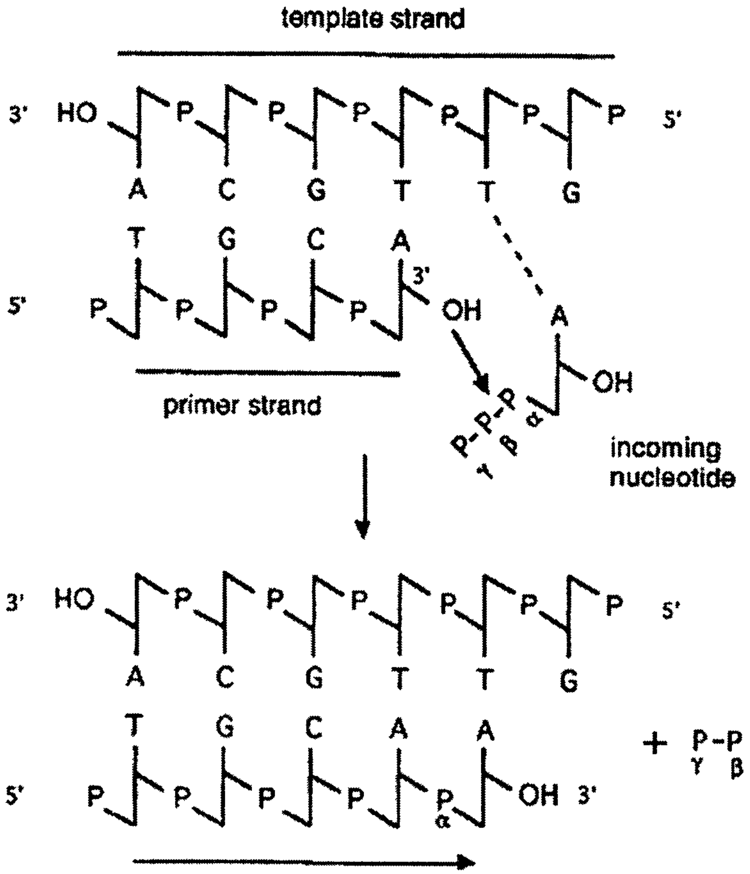

template strand
3' HO P P P P P P 5'
A C G T T G
T G C A
5' P P P P 3' OH
A
OH
primer strand
incoming nucleotide
3' HO P P P P P P 5'
A C G T T G
T G C A A
5' P P P P P α OH 3'
+ P-P γ β

Fig. 11.20 : Process of Nucleotide Attachment during 5' to 3' DNA Synthesis

DNA Synthesis is Semidiscontinuous

Since all known DNA polymerases can synthesize only in a 5' to 3' direction a problem arises in trying to replicate the two strands of DNA at a fork.

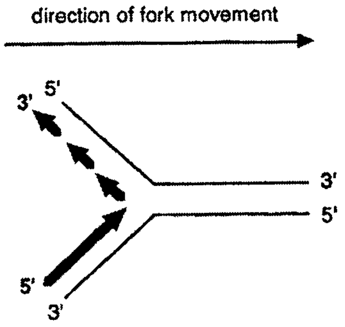


Fig. 11.21 : DNA synthesis as semidiscontinuous Process

The top strand must be discontinuously replicated in short stretches thus the replication of both parental strands is a semidiscontinuous process. The strand that is continuously sy thesized is called the leading strand while the strand that is discontinuously synthesized is called the lagging strand.

Leading Strand Synthesis (Fig 11.22)

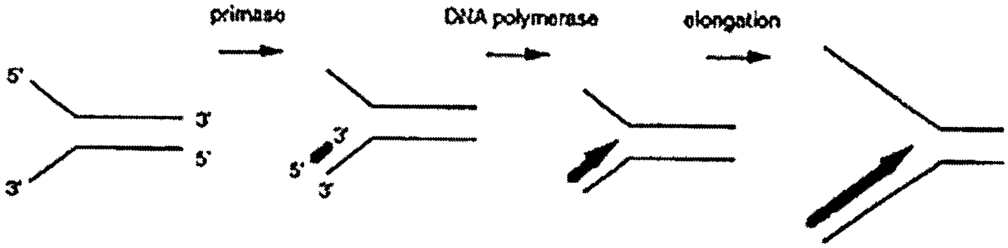


Fig 11.22 : DNA Synthesis at 5' to 3' Direction

DNA synthesis requires a primer usually made of RNA. A primase synthesizes the ribonucleotide primer ranging from 4 to 12 nucleotides in length. DNA polymerase then incorporates a dNMP onto the 3' end of the primer initiating leading strand synthesis. Only one primer is required for the initiation and propagation of leading strand synthesis.

Lagging Strand Synthesis (Fig 11.23)

Lagging strand synthesis is much more complex and involves five steps:

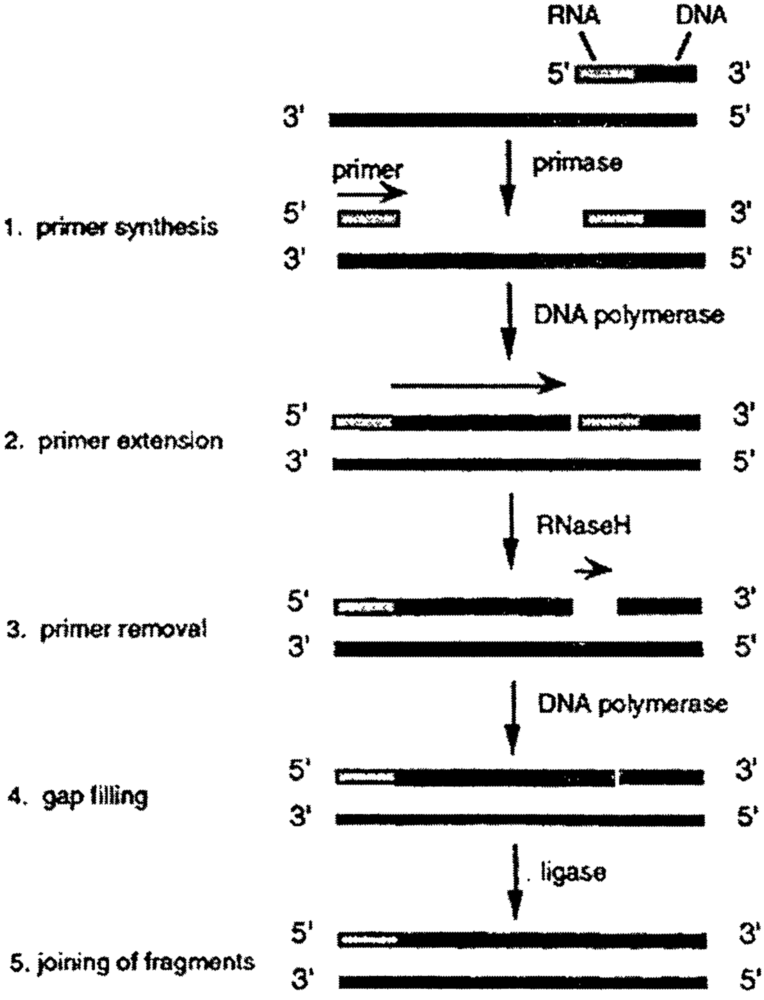

RNA
DNA
5'
3'
3'
5'
primer
primase
1. primer synthesis
5'
3'
3'
5'
DNA polymerase
2. primer extension
5'
3'
3'
5'
RNaseH
3. primer removal
5'
3'
3'
5'
DNA polymerase
4. gap filling
5'
3'
3'
5'
ligase
5. joining of fragments
5'
3'
3'
5'

Fig 11.23 : DNA Synthesis at 3' to 5' Direction

1. As the leading strand is synthesized along the lower parental strand the top parental strand becomes exposed. The strand is then recognized by a primase which synthesizes a short RNA primer.
2. DNA polymerase then incorporates a dNMP onto the 3" end of the primer and initiates lagging strand synthesis. The polymerase extends the primer for about 1,000 nucleotides until it comes in contact with the 5' end of the preceding primer. These short segments of RNA/DNA are known as Okazaki fragments (Fig 11.24).

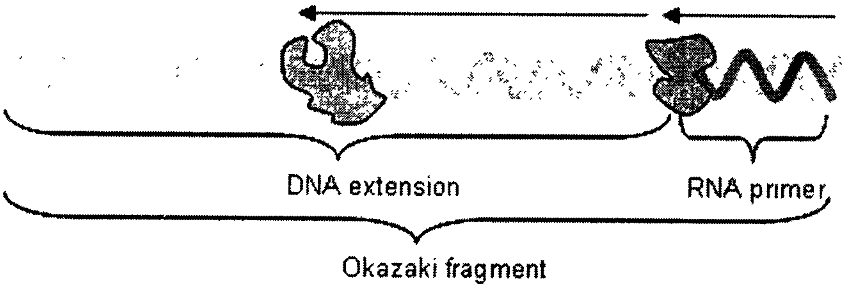


Fig 11.24 : DNA primase Adds RNA primers to ssDNA. These are extended to Okazaki fragments by DNA polymerase.

3. When the DNA polymerase encounters the preceding primer it dissociates. The RNA is then removed by a specialized DNA polymerase or by an enzyme called RNaseH. Ribonucleotides are then excised one at a time in a 5' to 3' direction. The RNaseH leaves a phosphate group at the 5' end of the adjoining DNA segment thus leaving a gap.
4. The gap is filled by a DNA polymerase which uses an Okazaki fragment as a primer.
5. The 3' hydroxyl group on the 3' nucleotide terminus is then covalently joined, using DNA ligase, to the free 5' phosphate of the previously made lagging segment.

Coordination of Leading and Lagging Strand Synthesis

Leading and lagging strand synthesis is thought to be coordinated at a replication fork. The two polymerases are held together by another set of proteins, τγ, which are near the fork that is being unwound and simultaneously primed by helicase-primase. Both polymerases are bound by a processivity factor, β. Upon completing an Okazaki fragment the lagging strand polymerase release the β factor and dissociates from the DNA (b in the Figure). The τγ complex then loads the new β factor/primer complex onto the lagging strand polymerase which initiates a new round..... (Fig 11.25)

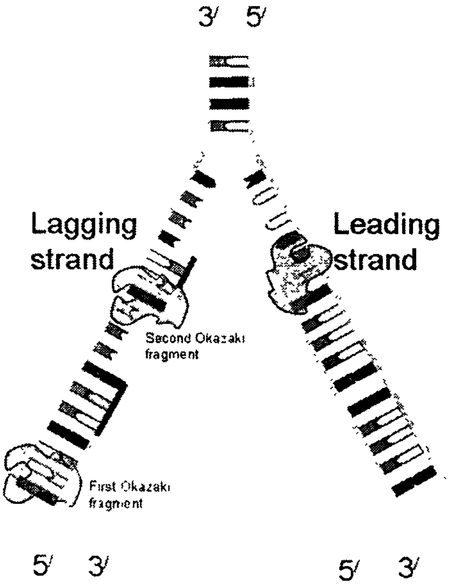


Fig 11.25 : Coordination of Leading and Lagging Strand of DNA synthesis

Telomerase

Leading strand synthesis can proceed all the way to the end of a chromosome however lagging strand synthesis can not. Consequently the 3' tips of each daughter chromosome would not be replicated.

Telomerase (also AKA telomere terminal transferase) extends the 3' ends of a chromosome by adding numerous repeats of a six base pair sequence until the 3' end of the lagging strand is long enough to be primed and extended by DNA polymerase (Fig 11.26).

Telomerase recognizes the tips of chromosomes also know as telomeres. The DNA sequences of telomeres have been determined in several organisms and consist of numerous repeats of a 6 to 8 base long sequence, [TTGGGG]n.

Telomerase consists of a protein and short RNA molecule that is complementary to the TTGGGG repeat in the telomere. This complementary RNA sequence base pairs with the telomere allowing Telomerase to add additional complementary bases to the 3' terminus of the telomere. The enzyme then translocates 6 bases towards the 3' end so that the RNA base pairs with the last two or three nucleotides that were just synthesized.. Telomerase then adds the next set of complementary nucleotides and repeats the cycle. Human Telomerase is not yet well characterized but is believed to follow a similar mechanism.

Telomeres have been found to progressively shorten in certain types of cells. These cells appear to lack Telomerase activity. When telomeric length shortens to a critical point the cell dies. Cells derived from rapidly proliferating tissues, such as tumours, have telomeres that are unusually long. This indicates that Telomerase activity may be necessary for the proliferation of tumor cells. Telomerase activity is found in ovarian cancer cells but not in normal ovarian tissue. Thus, it may be possible, once human Telomerase has been isolated and characterized, to develop anti-tumor drugs that function to inhibit telomerase activity.

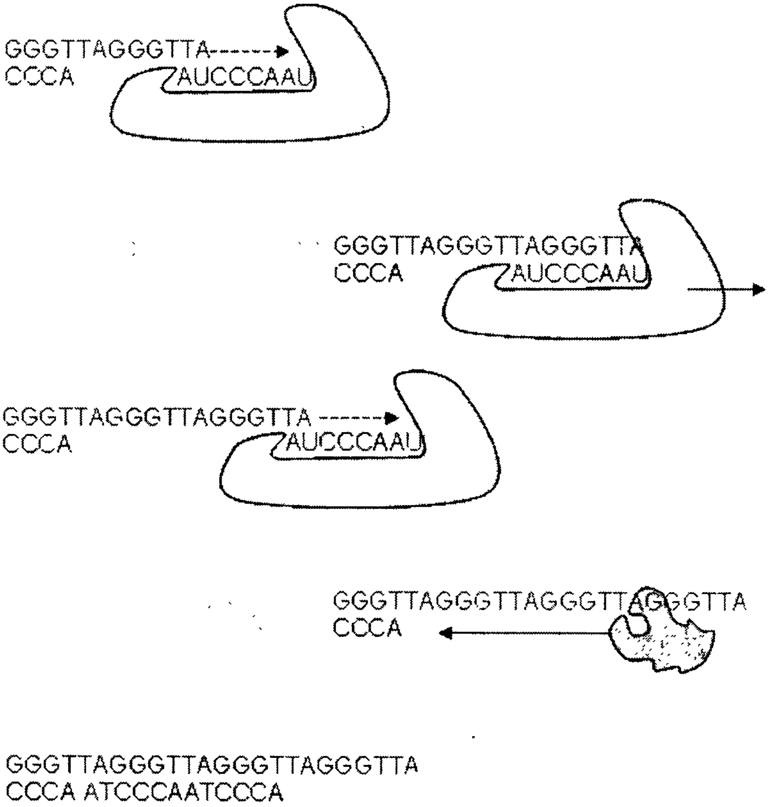

GGGTTAGGGTTA------►
CCCA
AUCCCAAU
GGGTTAGGGTTAGGGTTA
CCCA
AUCCCAAU
GGGTTAGGGTTAGGGTTA ------►
CCCA
AUCCCAAU
GGGTTAGGGTTAGGGTTAGGGTTA
CCCA
GGGTTAGGGTTAGGGTTAGGGTTA
CCCA ATCCCAATCCCA

Fig. 11.26 : Telomase extends Lagging strand by adding repeats of a six base pairs sequence at 3' ends

Thus, Telomerase is a reverse transcriptase with its own integral RNA template. It pairs with the telomere sequence and extends the ssDNA of the telomere.

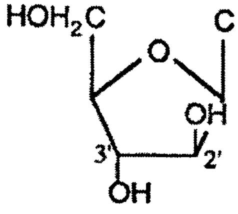
The telomerase RNA pairs with the telomere sequence and the enzyme then extends the telomere by RNA-templated DNA synthesis. DNA polymerase can then make (all but the end) double stranded.

Chemical Inhibitors of DNA Replication

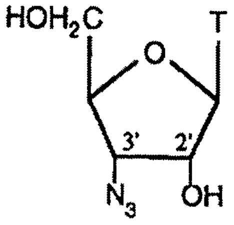
Some types of drugs function by inhibiting DNA replication.

Substrate Analogs: analogs of dNTP's which function as chain terminators can be incorporated into DNA. These analogs are usually either missing the 3' hydroxyl group or have a chemical group, other than hydroxyl, in the 3' position.

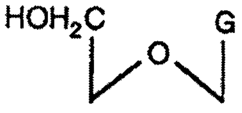
cytosine arabinoside



azidothymidine (AZT)



acyclovir



Cytosine Arabinoside: is an anticancer drug used to treat leukaemia.

Azidothymidine (AZT): was used as an anti-HIV drug that, while effective in tissue culture experiments, proved to be ineffective for treating HIV in humans.

Acyclovir: is an effective anti-herpes virus drug.

Intercalating Agents: are compounds with fused aromatic ring systems that can wedge (intercalate) between the stacked base pairs of DNA. This disrupts the structure of the DNA so that the replicative enzymes have difficulty in synthesizing DNA past the "intercalated" sites. Anthracycline glycosides and Actinomycin D are intercalators used to treat a variety of cancers.

DNA Damaging Agents: a variety of compounds such as Cisplatin, cause chemical damage to DNA and are used in the treatment of cancers.

Topoisomerase Inhibitors: Nalidixic acid and Fluoroquinolones are antibiotics used to inhibit bacterial topoisomerases.

QUIZ

Identify the letter of the choice that best completes the statement or Answers the question.

1. DNA is a polymer of:
   - A. nucleosides
   - B. fatty acids
   - C. deoxyribose sugars connected by phosphodiester bonds
   - D. nucleotides

Answer: D

2. Which of the following are pyrimidines?
   - A. adenine and cytosine
   - B. adenine and guanine
   - C. cytisine and thymine
   - D. cytosine and guanine

Answer: C

3. Which of the following are purines?
   - A. adenine and cytosine
   - B. adenine and guanine
   - C. adenine and thymine
   - D. cytosine and guanine

Answer: B

4. A nucleic acid was analyzed and found to contain 37 percent A, 16 percent G, 22 percent C, and 25 percent T. The nucleic acid must be:
   - A. single-stranded RNA
   - B. single-stranded DNA
   - C. double-stranded RNA
   - D. double-stranded DNA

Answer: B

5. The two polynucleotide chains in DNA are:
   - A. semidiscontinuous
   - B. semiconservative
   - C. parallel
   - D. antiparallel

Answer: D

6. In the semiconservative replication of DNA, progeny DNA molecules consist of:
   - A. one-half of the molecules with two parental strands and one-half of the molecules with two new strands
   - B. all molecules with interspersed parental and new segments
   - C. all molecules with one parental and one new strand

D. all molecules with two new strands

Answer: C

7. In the conservative model for replication of DNA, progeny DNA molecules consist of:
   - A. one-half of the molecules with two parental strands and one-half of the molecules with two new strands
   - B. all molecules with interspersed parental and new segments
   - C. all molecules with one parental and one new strand
   - D. all molecules with two new strands

Answer: A

8. What chemical group is found at the 5' end of a polynucleotide chain?
   - A. Phosphate
   - B. Nitrogen
   - C. Ribose
   - D. Hydrogen

Answer: A

9. What is the base sequence of the DNA strand that would be complementary to the 5' GGATCTGATCCAGTCA 3'single-stranded DNA molecule?
   - A. 3' CCTAGACTAGGACAGT 5'
   - B. 3' CCTAGAAAAGGTCAGT 5'
   - C. 3' CCTAGACTAGGTCAGT 5'
   - D. 3' CTTAGACTAGCTCAGT 5'

Answer: C

10. The percent of cytosine in a double-stranded DNA is 21. What is the percent of thymine in that DNA?
    - A. 21
    - B. 29
    - C. 50
    - D. 79

Answer: B

11. The percent of adenine in a double-stranded DNA is 38. What is the percent of cytosine in that DNA?
    - A. 50
    - B. 38
    - C. 12
    - D. 88

Answer: C

12. RNA is synthesized on a DNA template in a process called ______, which utilizes the enzyme _______
    - A. translation, RNA polymerase

B. transcription, DNA polymerase
C. transcription, RNA polymerase
D. replication, DNA polymerase

Answer: C

13. Which of the following is not a necessary component of translation?
A. anticodon
B. mRNA
C. ligase
D. amino acid

Answer: C

14. Which of the following is its complementary mRNA from DNA strand - GGACTGATT?
A. CCTGACTAA
B. CCUGACUAA
C. GGACTGATT
D. TTAGTCAGG

Answer: B

15. Amino acids are joined together into a protein chain by which of the following?
A. transfer RNA
B. DNA polymerase
C. hydrogen bonds
D. messenger RNA

Answer: A

16. Proteins contain ____ different amino acids, whereas DNA and RNA are composed of ___ different nucleotides
A. 20, 64
B. 3, 20
C. 4, 20
D. 20, 4

Answer: D

17. The base pair rules states that:
A. Replication is semiconservative
B. A pairs with T, G pairs with C
C. DNA is a double helix held together by hydrogen bonds
D. A pairs with G, T pairs with C

Answer: B

18. Once transcription has been completed, which of the following is not necessary for protein synthesis to occur?
A. tRNA
B. ribosomes

C. mRNA
D. DNA

Answer: D

19. Which site of the tRNA molecule binds to the mRNA molecule?
A. anticodon
B. codon
C. amino acid
D. 5 prime end

Answer: A

20. Okazaki fragments occur on the ___ and are bonded together by ______
A. leading strand, polymerase
B. mRNA, anticodons
C. lagging strand, ligase
D. tRNA, polymerase

Answer: C

21. How many different codons are possible?
A. 3
B. 20
C. 64
D. an infinite number

Answer: C

22. The strand of DNA that is to be complemented by the DNA polymerase is referred to as the:
A. primer
B. template
C. daughter strand
D. autoradiograph

Answer: B

23. What is the property of the chain-terminating nucleotides that enables them to terminate the polymerizing DNA chain?
A. They are radioactive.
B. They lack the nucleotide base.
C. They lack a 3'-hydroxyl.
D. They are fluorescent.

Answer: C

24. A student grows E. coli with 15NH4Cl for many generations so that 15N is incorporated into most purines and pyrimidines. The student then adds an excess of 14 NH_4Cl and grows the cells for 2 generations. The DNA is isolated from the cells and analyzed by CsCl centrifugation. What results should the student expect to see?
A. 1 band of DNA

B. 1 faint band of DNA and 1 strong band of DNA
C. 2 bands of equal intensity
D. 3 bands of equal intensity

Answer: C

25. DNA polymerase I requires which of the following in order to catalyze the synthesis of DNA?
A. a template DNA strand
B. all 4 deoxynucleotides
C. a primer
D. all of the above

Answer: D

26. DNA polymerase has which of the following functions?
A. 3' to 5' polymerase activity
B. 3' to 5' exonuclease activity
C. 5' to 3' polymerase activity
D. B and C

Answer: D

27. All of the following statements concerning telomeres are true except
A. Telomeres are tandemly repeated G-rich nucleotide sequences.
B. Telomeres are tandemly repeated AT-rich regions.
C. Telomeres are at the 3' end of chromosomes.
D. Telomerase uses RNA as its template.

Answer: B

28. In the central dogma of biochemistry, information is passed in the following order:
A. RNA - DNA - protein
B. protein - DNA - RNA
C. DNA - RNA - protein
D. RNA - protein - DNA

Answer: C

29. Most of the cellular transcription is performed by:
A. the ribosome
B. RNA polymerases
C. DNA polymerases
D. B and C

Answer: B

30. What is the main role of the s (sigma) factor in the E. coli RNA polymerase complex?
A. They stabilize the polymerase.
B. They unwind the DNA ahead of the transcription complex.
C. They recognize promoter sequences on DNA.

D. They help chain elongation.

Answer: C

31. How many classes of RNA polymerases are found in eukaryotes?
A. 1 B. 2 C. 3 D. 4

Answer: C

32. Which of the following is the lac repressor?
A. lacI B. lacZ C. lacY D. lacA

Answer: B

33. The primary importance of bacteria to mankind is
A. production of secondary metabolites
B. Production of enzymes
C. Production of chemicals
D. for food

Answer: B

34. DNA (Deoxyribonucleic acid) are
A. blocks of protein
B. large organic macromolecule
C. synthetic acid
D. Answer not above

Answer: B

35. DNA performs the following functions except
A. transmission of information from one cell generation to another
B. provide information for protein synthesis
C. help in digestion of protein
D. Answer not above

Answer: B

36. The structure of a DNA is
A. sphere
B. double helix
C. rod
D. circle

Answer: B

37. The function of the messenger RNA is to
A. enable protein synthesis
B. provide information for repairing damages cells
C. enable removal of used cells
D. Answer not above

Answer: A

38. The messenger RNA is produced in the
A. genes
B. blood

C. DNA
D. bone marrows

Answer: C

39. How many enzymes have been identified in the body
A. 100
B. 15,000
C. 3,000
D. 6,000,000

Answer: C

40. Proteases are used for the following except
A. Chillproofing of beer
B. Biomedical applications
C. Monitoring serum cholesterol levels
D. Detergents

Answer: C

41. Biotechnology started 6,000 years back with
A. use of tractors
B. fermentation of beer
C. importation of foreign materials
D. it did not start 6,000 years ago.

Answer: B

42. Genetically modified potatoes as being noticed to affect which of the following animals
A. lizards
B. poultry
C. rat
D. pigs

Answer: C

43. Genes are exchanged between plants via
A. air
B. pollen tube
C. anther
D. filaments

Answer: B

44. Thermal stability of lysosome has being increased with the concept of
A. DNA science
B. protein engineering
C. modern gene technology
D. Answer not above

Answer: B

45. Who discovered that genes was located on chromosomes
    - A. Thomas Hunt Morgan
    - B. Louis Pasteur
    - C. Gregor Mendel
    - D. James Beal

Answer: A

46. he first experimental hybrid corn was developed in
    - A. 1879
    - B. 1820
    - C. 1967
    - D. 1904

Answer: A

47. Pasteurization was developed in
    - A. 1861
    - B. 1890
    - C. 1209
    - D. 2004

Answer: A

48. In 1943 the organism used to show that DNA carries the cell's genetic information was a
    - A. fungi
    - B. algea
    - C. bacteria
    - D. human being

Answer: C

49. The hematopoietic stem cell was discovered in
    - A. 1961
    - B. 1903
    - C. 1814
    - D. 4000AD

Answer: A

50. The First release of genetically engineered microbes in field experiments was in
    - A. 1987
    - B. 1904
    - C. 40 AD
    - D. 2004

Answer: A

51. Dairy farming developed as far back as
    - A. 1803
    - B. 4000AD

C. 1143
D. 2004

Answer: B

52. Genetic transformation was discovered by
A. Jost coins
B. Griffith
C. Maclyn McCarty
D. Cesar Milstein

Answer: B

53. The T-cell receptor is also known as
A. the "holy grail" of immunology
B. block of "life"
C. river of refurblishment
D. Answer not above

Answer: A

54. Restriction endonucleases, such as EcoR1, cleave:
A. by removing purine bases from the polynucleotide chain.
B. by removing pyrimidines from the polynucleotide chain.
C. only when it is single-stranded.
D. by breaking phosphodiester linkages in double-stranded DNA.

Answer: D

55. What are the two most important discoveries that enabled biochemists to start determining the primary structure of nucleic acid polymers?
A. restriction endonucleases
B. diisofluorophosphate
C. polyacrylamide gel electrophoresis
D. A and C

Answer: D

56. When DNA strands of different length separate on a polyacrylamide gel, which of following occurs?
A. Larger fragments travel faster.
B. The electric field causes bands to break.
C. Smaller fragments travel faster.
D. The polyacrylamide fluoresces.

Answer: C

57. When doing a DNA sequencing reaction, what is the minimum number of parallel reactions needed to get a complete sequence?
A. 1 B. 2 C. 4 D. 5

Answer: C

58. What is the main difference between the Sanger dideoxy method and the Maxam-Gilbert sequencing method?

A. They are the same except the detection method is different.
B. One elongates and the other cleaves DNA.
C. The Maxam-Gilbert method uses a lot more sample.
D. b and c

Answer: B

59. In order to be able to isolate plasmids containing a foreign gene of interest, cloning vectors usually contain a "selectable marker." These selectable markers are often:
    A. inverted repeats
    B. antibiotic resistance genes
    C. origins of replication
    D. telomeres

Answer: B

60. Blunt-end ligation is used to join covalently DNA molecules lacking 3-prime or 5-prime overhangs. This technique often relies on which of the following enzymes to accomplish this covalent ligation?
    A. phage T4 DNA ligase
    B. EcoR1 restriction endonuclease
    C. the lacZ gene product
    D. DNA polymerase

Answer: A

61. cDNA libraries are constructed by:
    A. synthesizing DNA copies of foreign genes from plasmids
    B. using PCR to non-specifically amplify DNA sequences using universal primers
    C. synthesizing cDNA from purified cellular mRNA using reverse transcriptase
    D. using SP6 RNA polymerase to transcribe foreign DNA

Answer: C

62. DNA ligases are used to:
    A. cleave double-stranded DNA at specific restriction sites
    B. join the ends of foreign DNA to the free ends of cleaved plasmid DNA
    C. cleave single-stranded segments of DNA fragments to yield blunt ends
    D. convert DNA sequences to RNA sequences

Answer: B

63. In Southern blots, specific sequences of DNA are identified by:
    A. annealing with a labeled single-stranded DNA probe
    B. annealing with a labeled single-stranded RNA
    C. binding to radioactively labeled antibodies

D. transcribing the DNA in an appropriate expression vector

Answer: A

64. Screening plasmid-based genomic libraries often uses a technique termed "colony hybridization." This technique can employ autoradiographic films, which detect:

A. RNA copies of the DNA sequence of interest
B. radioactive probes that hybridize with complementary DNA
C. radioactive copies of the target DNA that have been amplified by PCR
D. any non-radioactive copies of the target DNA that contrast with the radioactive label on the host DNA

Answer: B

65. In addition to their chromosomal DNA, bacteria contain DNA molecules called _______________ , which can be transferred from cell to cell via conjugation.

A. germline genes B. heteroduplex DNA
C. phages D. plasmids

Answer: D

66. _______________ is the mode of the delivery of bacterial genes from one cell to another via defective phage particles.

A. Transduction B. Conjugation
C. Transformation D. Transfection

Answer: D

Complete each sentence or statement.

1. A cluster of CG sequences that often marks the beginning of a gene in a mammalian genome is a/an ________.

Answer: CpG island

2. A single-strand break in a double-stranded nucleic acid is a/an ________.

Answer: nick

3. ________ is a topological state of DNA in which the helix is underwound or overwound so that the molecule tends to coil up on itself.

Answer: Supercoiling

4. ________ is the disk-shaped complex of a histone octamer and DNA that represents the fundamental unit of DNA organization in eukaryotes.

Answer: Nucleosome

5. ________ are the short segments of DNA formed in the discontinuous lagging strand synthesis of DNA.

Answer: Okazaki fragments

6. In addition to a polymerase active site that synthesizes a new polynucleotide chain, many polymerases contain an additional active site that acts to ________ the polymerase.

Answer: proofread

7. ________ is the transcriptionally active, relatively uncondensed chromatin in a eukaryotic cell.

Answer: Euchromatin

8. A heritable variation in the level of expression of a gene according to its parental origin is called ________.

Answer: imprinting

9. An oligonucleotide that base-pairs with a template polynucleotide strand and is extended through template-directed polymerization is a/an ________.

Answer: primer

10. During DNA ________, the parental polynucleotide strands separate so that each can direct the synthesis of a complementary daughter strand resulting in two complete DNA double helices.

Answer: replication

11. ________ is a property of a motor protein or other enzyme that undergoes many reaction cycles before dissociating from its track or substrate.

Answer: Processivity

12. Reverse transcriptase is a DNA polymerase that uses ________ as its template.

Answer: RNA

13. A model for DNA replication in which DNA polymerase and associated proteins remain stationary while the DNA template is spooled through them is the ________.

Answer: factory model of replication

14. The ________ is the DNA strand that is synthesized as a series of discontinuous fragments that are later joined.

Answer: lagging strand

15. A highly condensed, nonexpressed eukaryotic DNA is ________.

Answer: heterochromatin

16. ________ is the progressive movement of a single-strand break in DNA through the actions of an exonuclease that removes residues followed by a polymerase that replaces them.

Answer: Nick translation

17. Replication fork is the point in a replicating DNA molecule where the two ________ strands separate in order to serve as templates for the synthesis of new strands.

Answer: parental

18. The DNA strand that is synthesized continuously during DNA replication is the ________.

Answer: leading strand

19. Discontinuous synthesis is a mechanism whereby the ________ of DNA is synthesized as a series of fragments that are later joined.

Answer: lagging strand

20. The mechanism of DNA duplication in which each new molecule contains one strand from the parent molecule and one newly synthesized strand is ________.

Answer: semiconservative replication

21. A silencer is a DNA sequence some distance from the transcription start site where a/an ________ of transcription may bind.

Answer: repressor

22. A segment of DNA that is synthesized from an mRNA template and which therefore represents a portion of the genome that is expressed is called a/an ________.

Answer: expressed sequence tag

23. A/An ________ is a prokaryotic genetic unit that consists of several genes with related functions that are transcribed as a single mRNA molecule.

Answer: operon

24. The nucleolus is the region of the eukaryotic nucleus where rRNA is processed and ________ are assembled.

Answer: ribosomes

25. A portion of a gene that is transcribed but excised by splicing prior to translation is a/an ________.

Answer: intron

26. A TATA box is a eukaryotic promoter element with the consensus sequence TATA located 10 to 27 nucleotides upstream from the ________ start site.

Answer: transcription

27. ________ are the small L-shaped RNAs that deliver specific amino acids to ribosomes according to the sequence of a bound mRNA.

Answer: tRNA

28. RNA molecules that direct the sequence-specific methylation of eukaryotic rRNA transcripts are ________.

Answer: small nucleolar RNA or snoRNA

29. A/An ________ is a unique sequence of nucleotides that encodes a polypeptide or RNA.

Answer: gene

30. A portion of a gene that appears in both the primary and mature mRNA transcripts is a/an ________.

Answer: exon

31. A/An ________ is a protein that binds at or near a gene so as to increase its transcription.

Answer: activator

32. ________ is the addition, removal, or modification of nucleotides in an RNA molecule that are necessary to produce a fully functional RNA.

Answer: RNA processing

33. Spliceosome is a complex of protein and ________ that carries out the splicing of immature mRNA molecules.

Answer: snRNA

34. A genomic DNA sequence that potentially codes for a protein is called a/an ________.

Answer: open reading frame

35. ________ are the RNA molecules that provide structural support for the ribosome and catalyze peptide bond formation.

Answer: Ribosomal RNA (rRNA)

36. A DNA or RNA sequence showing the nucleotides most commonly found at each position is a/an ________.

Answer: consensus sequence

37. A eukaryotic DNA sequence located some distance from the transcription start site, where an activator of transcription may bind, is a/an ________.

Answer: enhancer

38. A/An ________ is a set of eukaryotic genes whose expression is coordinated.

Answer: synexpression group

39. One of a set of eukaryotic proteins that are required for the synthesis of all mRNAs is ________.

Answer: general transcription factor

40. The ________ is the DNA sequence at which RNA polymerase binds to initiate transcription.

Answer: promoter

41. A cap is a 7-methylguanosine residue that is posttranslationally added to the 59' end of a eukaryotic ________.

Answer: mRNA

42. ________ is a DNA repair pathway that removes and replaces mispaired nucleotides on a newly synthesized DNA strand.

Answer: Mismatch repair

43. A mass of cells resulting from the uncontrolled proliferation of cancer cells is a/an ________.

Answer: tumor

44. The ligation process that repairs a double-stranded break in DNA is called ________.

Answer: end joining

45. A viral gene that interferes with the normal regulation of cell growth and contributes to cancer is a/an ________.

Answer: oncogene

46. The ________ is the phase of the cell cycle when mitosis occurs.

Answer: M phase

47. ________ is one mechanism for repairing damaged DNA by allowing a homologous segment to serve as a template for replacement of the damaged bases.

Answer: Recombination

48. The formation of new blood vessels is called ________.

Answer: angiogenesis

49. The rate enhancement resulting from transition state stabilization by the substrate rather than the enzyme is called ________.

Answer: substrate catalysis

50. ________ is programmed cell death that results from extracellular or intracellular signals and involves the activation of caspases that selectively degrade cellular structures.

Answer: Apoptosis

51. A DNA repair pathway in which a damaged single-stranded segment of DNA is removed and replaced with normal DNA is called ________.

Answer: nucleotide excision repair

52. The substitution of one base for another in DNA that arises from mispairing during DNA replication or from chemical alterations of existing bases is called ________.

Answer: point mutation

53. ________ means being expressed at a continuous, steady rate rather than induced.

Answer: Constitutive

54. The deoxyribose residue remaining after the removal of a base from a DNA strand is knows as a/an ________ site.

Answer: abasic

55. ________ is the process of developing cancer.

Answer: Carcinogenesis

56. ________ is the process by which a normal cell becomes a cancer cell.

Answer: Transformation

57. An agent that induces a mutation in an organism is a/an ________.

Answer: mutagen

58. The phase of the cell cycle in which DNA replication occurs is the ________.

Answer: S phase

59. ________ is the normal cellular analog of an oncogene, whose mutation may contribute to cancer.

Answer: Proto-oncogene

60. A gene whose loss or mutation may lead to cancer is a/an ________.

Answer: tumor suppressor gene

61. Base excision repair is a DNA repair pathway in which a damaged base is removed by an enzyme known as a/an ________ so that the resulting abasic site can be repaired.

Answer: glycosylase

“This Page is Intentionally Left Blank”

Chapter 12

Amino Acid and Protein Metabolism

Protein metabolism is a key physiological process in all forms of life. Proteins are converted to amino acids and then catabolised. The complete hydrolysis of a polypeptide requires mixture of peptidases because individual peptidases do not cleave all peptide bonds. Both exopeptidases and endopeptidases are required for complete conversion of protein to amino acids.

Protein Catabolism

Peptidases

Exopeptidases

Exopeptidases hydrolyse the polypeptides either from the carboxyl terminus (carboxypeptidases) or from the amino terminus (amino peptidase). Some of the important exopeptidases and their sources are given in below

Exopeptidase	**Source**
Carboxypeptidase A	Bovine pancreas
Carboxypeptidase B	Bovine Pancreas
Carboxypeptidase C	Citrus leaves
Amino peptidase M	Porcine kidney
Leucine amino peptidase	Porcine kidney

Endopeptidases

Enzymes, which hydrolyse the internal peptide bonds, are called as endopeptidases. Endopeptidases have side chain requirements for the residues flanking the scissile peptide bond. (Peptide bond to be cleaved). The important endopeptidases and their sources are listed below

Enzyme	**Source**
Trypsin	Bovine pancreas
Chymotrypsin	Bovine pancreas
Pepsin	Bovine gastric
Elastase	Bovine pancreas
Papain	Papaya latex

Trypsin

It is a proteolytic enzyme, present in the intestine in its inactive form (zymogen), trypsinogen. Trypsinogen is converted into its active form, trypsin, by enteropeptidase, a specialized proteolytic enzyme secreted by intestinal cells. Some free trypsin formed also catalyses the conversion of trypsinogen into trypsin. Trypsin can also convert chymotrypsinogen and procarboxypeptidase into chymotrypsin and carboxypeptidase, respectively. Trypsin has different amino acid specificity when compared with other proteolytic enzymes. Trypsin hydrolyses those peptide bonds whose carboxyl groups are contributed by Lys or Arg residues and if the next residue is not proline. The number of smaller peptides resulting from trypsin action is equal to the total number of Arg and Lys residues in the protein plus one.

Papain

Papain is widely used in brewing industry, for tenderization in meat industry, fish, food, laundry, detergents, pharmaceutical and allied industries. Papain consists of a single polypeptide chain of 212 amino acid residues, cross-linked by four disulphide bonds. The active site of papain contains a cysteine residue whose sulphhydryl group is required for catalysis. It has a broad specificity for peptide bonds with wide pH range.

Pepsin

The precursor of pepsin is pepsinogen (MW 40,000) and is converted into active pepsin in the gastric juice by the enzymatic action of pepsin itself. In this conversion, 42 amino acid residues are removed from the amino-terminal end of the polypeptide chain. The portion of the molecule that remains intact is enzymatically active pepsin of molecular weight 33,000 Dalton. Pepsin hydrolyses proteins at peptide bonds on the amino terminal side of tyrosine, phenylalanine and tryptophan and converts it into a mixture of smaller peptides.

Chymotrypsin

Chymotrypsin is secreted from the pancreas in the zymogen form as chymotrypsinogen and it is converted to the active form by trypsin. It reacts with the substrates protein, proteoses and peptones cleaving the peptide bonds whose carboxyl groups are furnished by aromatic amino acids.

Amino Acid Metabolism

The amino acids not only function as energy metabolites but also used as precursors of many physiologically important compounds such as heme, bioactive amines, small peptides, nucleotides and nucleotide coenzymes. In normal human beings about 90% of the energy requirement is met by oxidation of carbohydrates and fats. The remaining 10% comes from oxidation of the carbon skeleton of amino acids. Since the 20 common protein amino acids are distinctive in terms of their carbon skeletons, amino acids require unique degradative pathway. The degradation of the carbon skeletons of 20 amino acids converges to just seven metabolic intermediates namely.

- Pyruvate
- Acetyl CoA
- Acetoacetyl CoA
- α-Ketoglutarate
- Succinyl CoA
- Fumarate
- Oxaloacetate

Pyruvate, α-ketoglutarate, succinyl CoA, fumarate and oxaloacetate can serve as precursors for glucose synthesis through gluconeogenesis. Amino acids giving rise to these intermediates are termed as glucogenic. Those amino acids degraded to yield acetyl CoA or acetoacetate are termed ketogenic since these compounds are used to synthesize ketone bodies. Some amino acids are both glucogenic and ketogenic (For example, phenylalanine, tyrosine, tryptophan and threonine)

Catabolism of Amino Acids

Protein catabolism and anabolism are often out of sync - either no additional protein is needed or the amino acid composition of the synthesized proteins is not identical to the protein being hydrolyzed. Neither protein nor amino acids are stored as such. Thus organisms must frequently degrade excess amino acids. Two paths may be available: deaminate the unneeded amino acids and breakdown the carbon skeletons for energy or for storage as fat or carbohydrate and eliminate the nitrogen; or the nitrogen may be transferred to another carbon backbone to make a needed amino acid.

Nitrogen can be eliminated in a variety of forms, depending on the physiological conditions experienced by the organism:

- **Ammonia** and/or ammonium ion. This occurs to some extent in all organisms. However ammonia is quite toxic so it must be kept at low concentrations in the body. Thus ammonia is used as the **major** excretory product in organisms with an essentially unlimited amount of water available to dilute it: plants, fish, many amphibians.
- **Urea**. Animals with readily available water (for drinking) use urea as their major nitrogenous waste product. Urea is non-toxic at low (<1 M) concentrations and is extremely soluble (>10 M). It is thus an excellent waste in liquid form. Used by mammals. (Lungfish have a variable

environment, water to mud, and switch back and forth between ammonia and urea as appropriate!).

- **Uric acid**. Animals living in deserts, environmental or physiological, use uric acid. Uric acid is quite insoluble, therefore water is not needed to excrete it. Unfortunately insolubility also means it has a tendency to crystallize out in joints etc. (e.g., small amounts in humans can lead to gout). Uric acid is thus used by reptiles and birds, including species living in or near water. The problem here is these animals all start life in a "physiological desert" within their eggs. Many birds of course also have the "migration problem" - they must fly immense distances and cannot afford to carry excess water (weight). (Spiders use an even more insoluble compound, xanthine).

The important reaction commonly employed in the breakdown of an amino acid is always the removal of its α-amino group. The product ammonia is excreted after conversion to urea or other products and the carbon skeleton is degraded to CO2 releasing energy. The important reaction involved in the deamination of amino acids is

- Transamination.
- Oxidative deamination.
- Non oxidative deamination.

Transamination

There are three main transaminases or **Amino transferases**, all requiring Pyridoxal-P as a cofactor:

- Glutamate aminotransferase (third most active in liver): Amino acid + 2-Oxoglutarate (α-kitoglutarate) $\rightleftharpoons$ 2-Oxoacid + Glutamate
- Alanine aminotransferase (second most active in liver): Alanine + 2-Oxoglutarate (α-kitoglutarate) $\rightleftharpoons$ Pyruvate + Glutamate
- Aspartate aminotransferase (most active in liver): Aspartate + 2-Oxoglutarate (α-kitoglutarate) $\rightleftharpoons$ Oxalacetate + Glutamate

Most amino acids are deaminated by transamination reaction catalysed by aminotransferases or transaminases. The α-amino group present in an amino acid is transferred to an α-keto acid to yield a new amino acid and the α-keto acid of the original amino acid. The predominant amino group acceptor is α-keto glutarate.

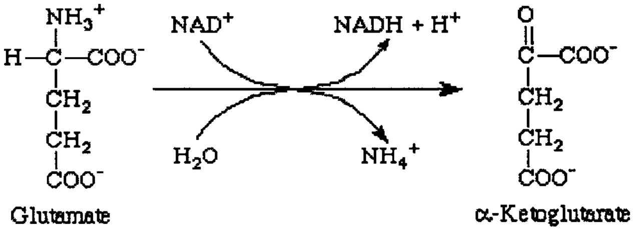
Pyridoxal phosphate, the coenzyme of pyridoxine (vitamin B6) plays an important role in these reactions. Amino transferase reactions occur in two stages.

Pyridoxal phosphate is covalently attached to the amino transferases via a Schiff's base linkage formed between the aldehyde group of pyridoxal phosphate and the epsilon amino group of lysine residue of the enzyme. Pyridoxal phosphate is converted to pyridoxamine phosphate. In the second stage, the amino group attached to pyridoxamine phosphate is transferred to a different keto acid to yield a new amino acid and releases pyridoxal phosphate

Oxidative Deamination

Transamination does not result in net deamination, since one amino acid is replaced by another amino acid. The function of transamination is to funnel the amino nitrogen into one or a few amino acids. For glutamate to play a role in the net conversion of amino groups to ammonia, a mechanism for glutamate deamination is needed so that α-ketoglutarate can be regenerated for further transamination. The generation is accomplished by the oxidative deamination of glutamate by glutamate dehydrogenase. Glutamate is oxidatively deaminated in the mitochondrion by glutamate dehydrogenase. NAD+ or NADP+ functions as the coenzyme. Oxidation is thought to occur with the transfer of a hydride ion from glutamate's α carbon to NAD(P)+ to form α-iminoglutarate, which is then hydrolysed to α-ketoglutarate and ammonia. The ammonia produced is then converted to urea in mammals.

Most ammonia results from the deamination of a single amino acid: glutamate, via the **Glutamate dehydrogenase** reaction:



This enzyme is somewhat unusual in that it can use either NADH or NADPH. Glutamate DH is activated by ADP and inhibited by GTP *in vitro* so they may regulate it *in vivo*. Of course these nucleotides would not provide a highly sensitive regulation, activity would only loosely follow energy charge.

How are the other amino acids deaminated? Most are transaminated, transferring their N to make glutamate:

$$\text{amino acid} + \alpha\text{-ketoglutarate} \rightleftharpoons \alpha\text{-keto acid} + \text{glutamate}$$

Amino Acid Oxidase

Two non-specific amino acid oxidases namely, L-amino acid and D-amino acid oxidases catalyse the oxidation of L and D-amino acids utilizing FAD as their coenzymes.

Amino acid + FAD + H2O ------------→ α-Keto acid + NH3 + FADH2

Non-oxidative Deamination

Amino acids such as serine and histidine are deaminated non-oxidatively

The other reactions involved in the catabolism of amino acids are decarboxylation, transulfuration, desulfuration, dehydration etc. The decarboxylation process is important since the products of decarboxylation reactions give rise to physiologically active amines.

Decarboxylation

The enzymes, amino acid decarboxylases are pyridoxal phosphate- dependent enzymes. Pyridoxal phosphate forms a Schiff's base with the amino acid so as to stabilise the α-carbanion formed by the cleavage of bond between carboxyl and α-carbon atom. The physiologically active amines epinephrine, nor-epinephrine, dopamine, serotonin, γ-amino butyrate and histamine are formed through decarboxylation of the corresponding precursor amino acids

Urea Cycle

Location : Liver, minimal activity Kidney

Urea is synthesized from ammonia, carbon dioxide, and aspartate nitrogen using

Kreb's Urea Cycle:

Two ATP's are required to synthesize carbamyl phosphate, the first to activate bicarbonate, the second to provide the activated phosphate on the carbamyl-P itself. Carbamyl-P is itself a high-energy compound with a mixed anhydride bond much like we saw in 1,3-*bis* PGA. The carbamyl group is then transferred onto ornithine which acts as a carrier for the growing urea molecule. Addition of the aspartate nitrogen requires two additional ATP equivalents to drive the condensation to argininosuccinate. Lysis results in the formation of a fumerate and arginine, which is then hydrolyzed to give our product, Urea, and regenerate the ornithine carrier (Fig 12.1).

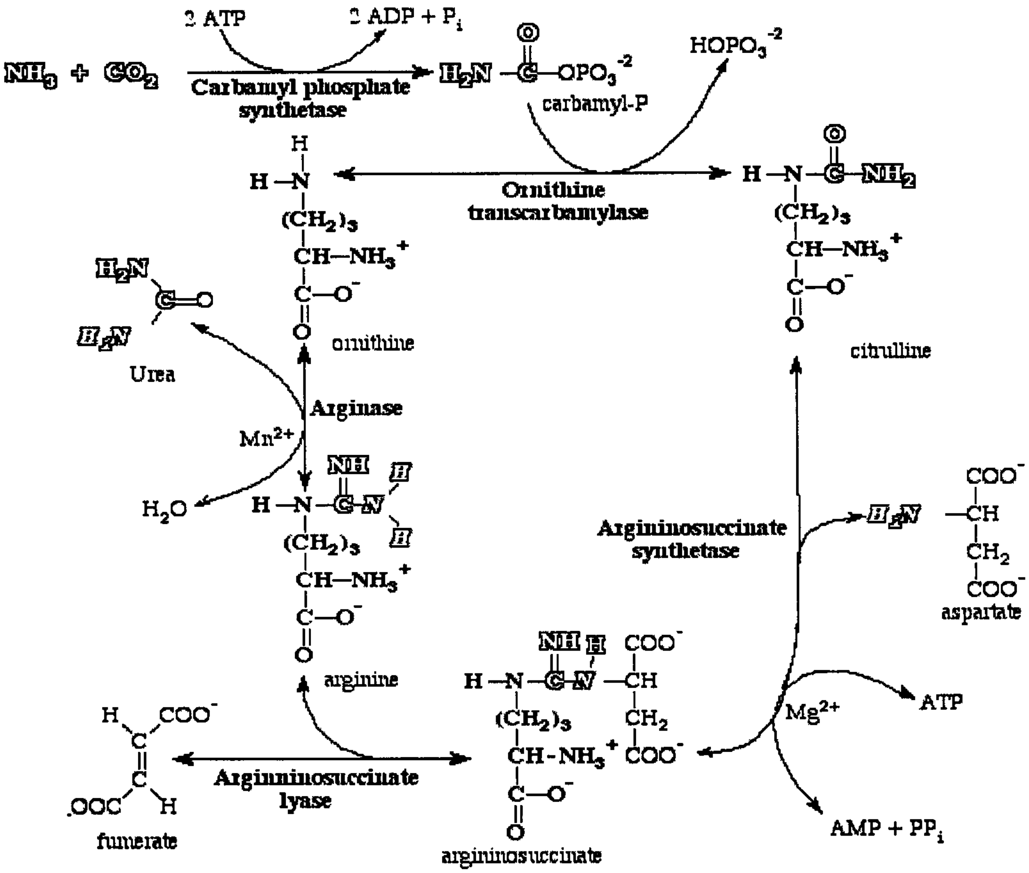

2 ATP
2 ADP + P_i
NH_3 + CO_2
Carbamyl phosphate synthetase
carbamyl-P
$HOPO_3^{-2}$
Ornithine transcarbamylase
ornithine
citrulline
Urea
Arginase
Mn^{2+}
H_2O
arginine
Argininosuccinate synthetase
aspartate
Mg^{2+}
ATP
AMP + PP_i
argininosuccinate
Argininosuccinate lyase
fumerate

Fig. 12.1 : Urea Cycle

[1] **Carbamoyl Phosphate Synthetase I (CPS I)**: provides the substrate, carbamoyl phosphate, for the urea cycle.

Allosteric Activation: N-Acetylglutamate via N-Acetylglutamate Synthase

Glutamate + Acetyl CoA ----> N-Acetylglutamate + CoA

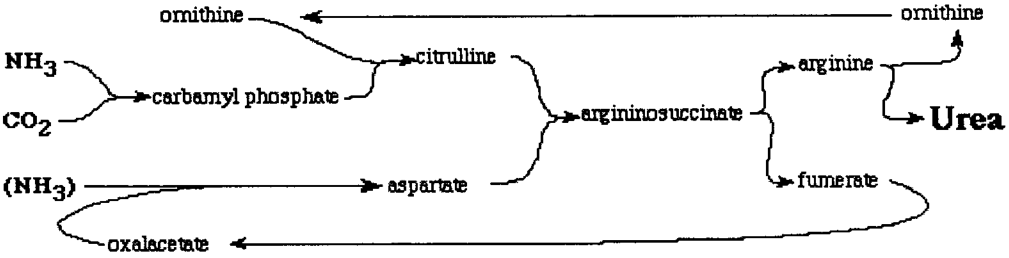
[2] **Ornithine Transcarbamoylase** (OTC): citrulline is exported from the mitochondria to the cytosol in exchange for ornithine.

[3] **Argininosuccinate Synthetase**: uses two high energy phosphate bonds

[4] **Argininosuccinate Lyase**: removes all but the amino group from Asp, fumarate is recycled back to Asp via malate and OAA in mitochondria.

[5] **Arginase**: urea is produced and ornithine is regenerated.

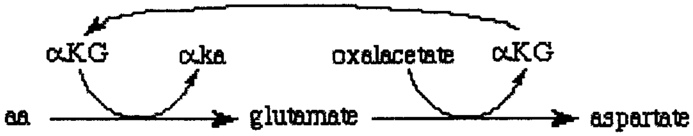
A total of four ATP equivalents has been consumed. How many ATP equivalents are then required to convert the nitrogen of two amino acids into urea? The flow diagram supplied below may help in this calculation:


ornithine
ornithine
NH3
CO2
carbamyl phosphate
citrulline
argininosuccinate
arginine
Urea
(NH3)
aspartate
fumerate
oxalacetate

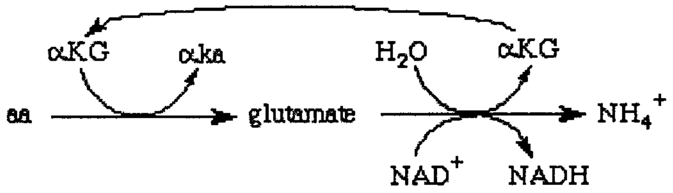
To solve this problem we can first note the four ATP equivalents consumed in the biosynthesis of one urea from two amino acids. But the fumerate produced must be taken back to regenerate the oxalacetate used to pick up the nitrogen from one amino acid):

Fumerate + $H_2O \longrightarrow$ Malate; Malate + $NAD^+ \longrightarrow$ oxaloacetate + NADH + H^+

This will provide an NADH via malate DH. This is equivalent to 2.5 ATP's, so we have -4 + 2.5 = -1.5. Next, while the second nitrogen enters via two transaminations through aspartate:


αKG
αka
oxalacetate
αKG
aa
glutamate
aspartate

The ammonia may also produced from the first amino acid via glutamate using Glutamate DH,


αKG
αka
H_2O
αKG
aa
glutamate
NH_4^+
NAD^+
NADH

This also produces an NADH, providing another 2.5 ATP's. thus the final tally is -4 + 2.5 + 2.5 = +1 ATP to produce one urea from two amino acid nitrogens. Of course for mammals this does not take into account the physiological costs of excreting the urea, which can be significant.

Overall Reaction:

$NH_3 + CO_2 + 3\ ATP + Asp \text{ ----> } Urea + Fumarate + 2\ ATP + AMP + 2\ P_i + PP_i$

Urea Cycle Compartmentation

The enzymes involved in the conversion of amino acid nitrogen into urea occur in the cytosol and in the mitosol and involve three different catalytic systems: the Urea Cycle, The TCA cycle, and a transamination cycle, you might call them "Kreb's Tricycle."

Note the involvement of two antiports to move intermediates across the inner mitochondrial membrane: the malate: aspartate antiport, and the citrulline:ornithine antiport. Note also that nitrogen is incorporated in the mitosol (Transamination, Carbamoyl-P synthesis), whereas the final product, urea is released in the cytosol.

Nitrogen Excretion:

The body removes nitrogen waste in a variety of forms (Table 12.1).

Table 12.1 : Various Types of Nitrogenous Compounds excreted from the body.

Compound	Source
Urea	Amino aicd catabolism, ammonia formation
Urobilinogen	Heme breakdown
N-Methylhistidine	Myofibrillar protein breakdown
Cratinine	Muscle creatine
Amino acids	Tissue proteins, pyrimidines
Uric acid	Purine breakdown

Clinical Correlate:

Aquired Hyperammonemia: may result from cirrhosis of the liver and is only cured by a liver transplant.

Inherited Hyperammonemia

Cause: deficiencies of urea cycle enzymes, almost exclusively seen in children.

Symptoms: severity depends on proximity of defect to point of entry of ammonia into the cycle (CPS I step) can be life-threatening to no symptoms at all.

A total deficiency of an enzyme is usually fatal or results in severe mental retardation.

Causes of Neurologic Damage:

(1) ammonia reacts with α-ketoglutarate to form glutamate thus interfering with ATP production in the TCA cycle

(2) excess glutamate undergoes successive amination to glutamine then α-ketoglutaramic acid, a neorotoxic cmpd.

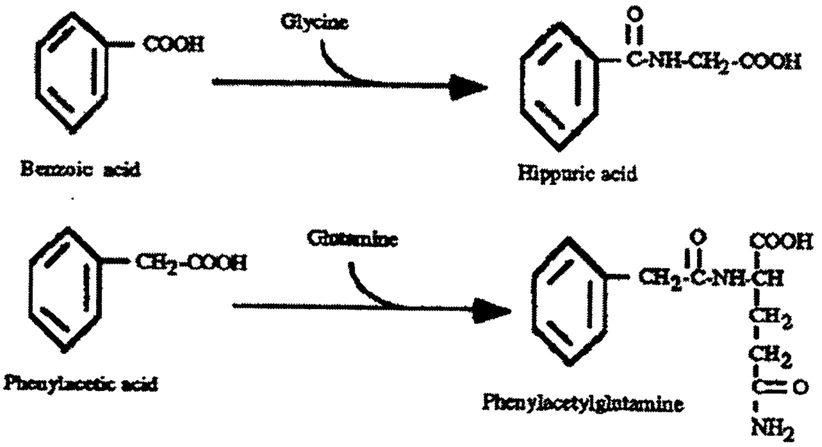
(3) The huge increases of a single amino acid in the blood result in limited availability of others within the brain, thus reducing rates of protein synthesis.

Treatment:

(1) Dietary restriction of proteins.

(2) Organic Acid Conjugation: Benzoic and Phenylacetic Acid

Benzoic Acid: conjugated with Gly forms hippuric acid which is readily excreted in the urine. Gly can continue to be synthesized from CO_2 and NH_3.


COOH
Glycine
C-NH-CH2-COOH
Benzoic acid
Hippuric acid
CH2-COOH
Glutamine
CH2-C-NH-CH
COOH
CH2
CH2
C=O
NH2
Phenylacetic acid
Phenylacetylglutamine

Phenylacetic Acid: conjugated with Gln forming phenylacetylglutamine which is readily excreted. Gln can be synthesized via the Glutamate DH rxn.

Alanine Cycle

Much of the nitrogen is carried between the tissues and the liver by the **Alanine Cycle.** (Fig 12.2)

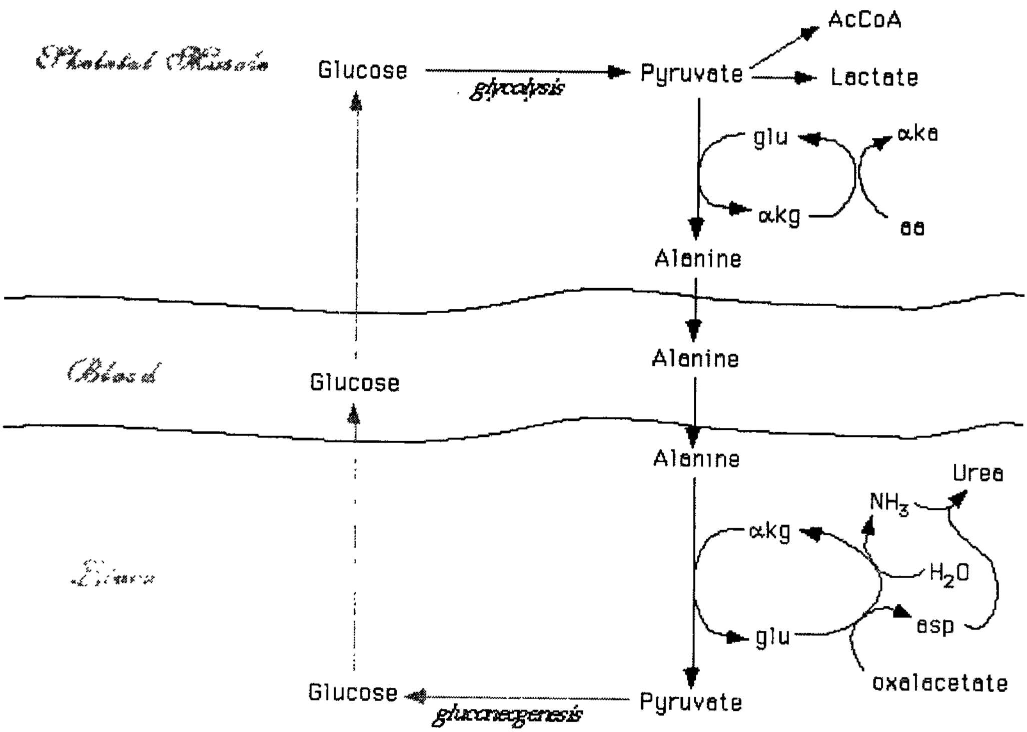

Skeletal Muscle
Glucose
glycolysis
Pyruvate
AcCoA
Lactate
glu
αka
αkg
aa
Alanine
Blood
Glucose
Alanine
Liver
Alanine
Urea
NH_3
αkg
H_2O
asp
glu
oxaloacetate
Glucose
gluconeogenesis
Pyruvate

Fig. 12.2 : Alanine Cycle in different Tissues

A similar, but more complex cycle involves glutamate/glutamine taking nitrogen from the muscle to the intestine where the glutamine is catabolized and the nitrogen goes to alanine which then goes to the liver. Glucose can then go to the muscle and be converted to glutamate via Glycolysis and the TCA cycle. We will not look at this more complex system.

Catabolism of Amino Acid Carbon Skeletons

Amino acids can also be categorized as being **glucogenic** (can be used in Gluconeogenesis) or **ketogenic** (cannot be used in Gluconeogenesis). Most amino acids can be at least partially used in glucose synthesis. For example ilu, tyr and phe are partially glucogenic and partially ketogenic (some carbons go to acetyl-CoA, while the rest go to TCA intermediates), while leu and lys are fully ketogenic.

Let begin by looking at the catabolism of amino acids by groups: 3-C (feed into pyruvate), 4-C (feed into oxalacetate), and 5-C (feed into glutamate).

3-C Amino acid's: **Ser** and **ala** are converted in single step processes to pyruvate. **Cys** is converted after first oxidizing and removing sulfur as sulphate.

(Threonine, glycine and part of tryptophan can also breakdown to pyruvate, but we will look at other paths).

4-C Amino acid's: **Asn** is hydrolyzed in one step to **aspartate**, which in turn is transaminated in one step to oxalacetate. **Threonine** feeds into the TCA cycle through succinyl-CoA instead of oxalacetate. Thr is first deaminated via a dehydratase as seen earlier, then decarboxylated by Pyruvate DH Complex to give propionyl-CoA, which is then transformed via a series of steps to give succinyl-CoA.

5-C Amino acid's: Five aa's feed into **glutamate** which in turns feeds into the TCA cycle at 2-oxo-glutarate.

- **Histidine** (his) is first deaminated, then the ring is opened and the formamino group is then donated to the one-carbon pool (see later). Two of these reactions are irreversible so his is essential.
- **Proline** (pro) is first oxidized and then hydrolyzed to open the ring and give glutamaldehyde which is oxidized to give glutamate. Note that the glutamaldehyde tends to spontaneously refold to the ring, which can then be reduced to synthesize proline (note the two opposing redox reactions are each irreversible). It is thus not essential.
- **Glutamine** (gln) is hydrolyzed in one step to glutamate.
- **Arginine** (arg) is hydrolyzed to ornithine by arginase from the urea cycle.
    - **Ornithine** is then transaminated to glutamaldehyde as seen with proline.
    - Arginine is essential for infants because the arginase removes essentially all of the arg made in the urea cycle, and glutamaldehyde's tendency to cyclize means it cannot be effectively synthesized from glutamate. (Bacteria use a blocking group to stop cyclization at this stage).

Branched Chain Amino Acids: valine (**val**), leucine (**leu**), and isoleucine (**ilu**). The metabolism of each of these three amino acids begins with the same theme: transaminase; DH Complex; *beta*-oxidation. Due to the irreversible nature of the DH Complex all three are essential.

- In the case of **ilu** this pattern leads to propionyl-CoA without modification. Thus you already know the chemistry to breakdown isoleucine. You should be able to show a pathway whereby the carbon in isoleucine is used to make glucose. Note the tissue distribution of reactions and transport.

- **Val** goes through the first two steps of *beta*-oxidation after which its structure dictates different reactions to reach propionyl-CoA.

Metabolic Fates of Amino Acids: (Fig 12.3)

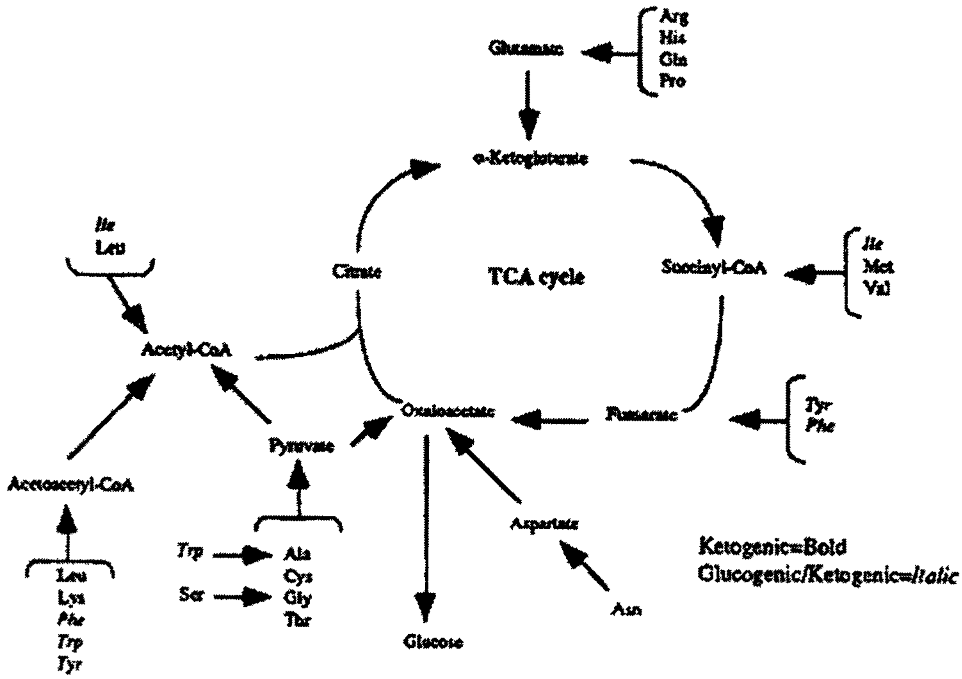

Glutamate
Arg
His
Gln
Pro
α-Ketoglutarate
Ile
Leu
Citrate
TCA cycle
Succinyl-CoA
Ile
Met
Val
Acetyl-CoA
Oxaloacetate
Fumarate
Tyr
Phe
Pyruvate
Acetoacetyl-CoA
Aspartate
Ketogenic=Bold
Glucogenic/Ketogenic=Italic
Leu
Lys
Phe
Trp
Tyr
Trp
Ser
Ala
Cys
Gly
Thr
Glucose
Asn

Fig. 12.3 : Schematic Presentation and Amino Acids Metabolism

Protein Synthesis

The genes tell the cell how to make proteins via transcription.

- They coined the phrase "One gene, one enzyme."
- By controlling enzymes genes control what kind of chemistry goes on in cells.
- Not all proteins are enzymes- some are structural.

The genes have a triplet code

- Genes must have a code specifying how proteins should be made.
- Nature uses 3 base words or "codons" for the genetic code.
    - Some amino acids have more than 1 codon.
    - A gene must have a starting point and an ending point.

  - 3 codons are stop codons (ATT, ATC and ACT).
  - There is 1 start codon, which also codes for the amino acid methionine (TAC).
- The genetic code was determined by using artificial RNA molecules (see below) to see what proteins they coded for.

The genetic code is universal

- Apparently, all species on earth use the same genetic code.
- A pea plant can translate human genes and vice versa.
- This points to a common origin of life on earth.
- The universal code is very useful in the biotech industry- we can make human proteins in other animals and plants.
- Some minor exceptions to the universal code are found in mitochondrial & chloroplast DNA and in a few protozoa.

When proteins are made a "working copy" of the gene is made from RNA

- DNA does not leave the nucleus, but proteins are made on ribosomes in the cytosol.
- The code message must be sent from the nucleus to the cytosol.
- When a protein is to be made a working copy of the gene is made out of RNA; this is called transcription.
- The DNA strands act as templates for making RNA copies of genes.
- The RNA copy is complementary to the DNA copy and the base T is replaced by U.

-T-C-A-T-T-G-T-G-C-A-A-C- DNA gene

-A-G-U-A-A-C-A-C-G-U-U-G- m-RNA copy

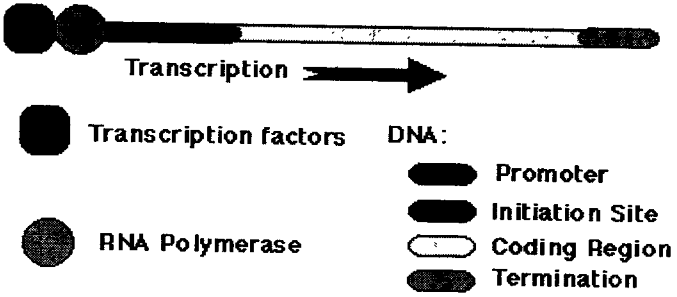
- The RNA copy of the code is called messenger RNA or m-RNA.
- This arrangement keeps the DNA master copy of the gene in the nucleus where it is protected.
- The working copy in RNA is made only when it is needed and is usually rapidly broken down.

Messenger RNA is made by RNA polymerase (Transcription)

- The RNA copy of DNA is made by an enzyme, RNA polymerase.
- Only the coding strand of DNA is transcribed.

- RNA polymerase binds to promoter region, just upstream from the coding section of the gene (TATA box).
- Protein transcription factors aid the binding of RNA polymerase, control RNA synthesis.
- Gene is transcribed:
  - From an initiation site (about 25 bases downstream from the polymerase binding site).
  - To a termination site (commonly AATAAA).
  - **Transfer of genetic information is** (Transcription is from the 5' to the 3' end almost).

Gene Structure



Always: DNA ⟶ RNA ⟶ Protein

- Genetic information is stored as DNA, transcribed to RNA and translated to protein:
  - DNA -> RNA -> protein
- Information does not flow in the reverse direction.
  - One exception: retroviruses store genetic information as RNA.
  - When they infect a cell they make a DNA copy of their RNA, using an enzyme, reverse transcriptase.

Genes have two types of DNA: Exons and Introns

- Eukaryotic cells have split genes.
- Non-coding sequences called introns are inserted in the middle of genes.
  - Prokaryotic genes seldom have introns: they are not split.
  - Prokaryotes have less room to store their genetic information.
- Coding sections are called exons.
- Both types of DNA are transcribed into m-RNA.
- Later the introns are cut out by complexes called spliceosomes.
  - Exons are ligated together.

- The messenger RNA is also given a cap and a polyadenine tail before being sent to the cytosol. (Fig 12.4)
    - GTP cap may help attach m-RNA to ribosome.
    - A long polyA tail increases the half life of the m-RNA.

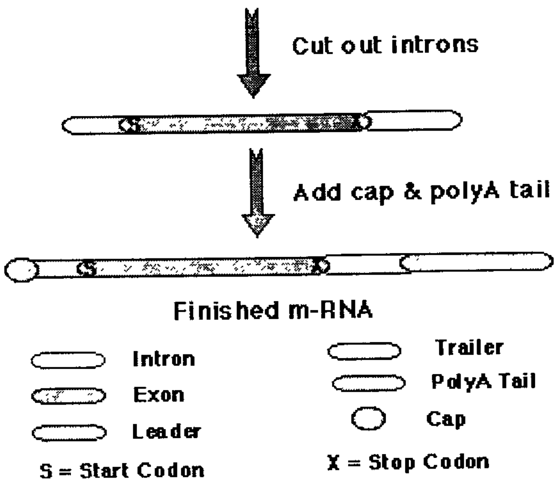


Fig. 12.4 : An Overview of mRNA Biosynthesis

Synthesis and Destruction of RNA is used to control metabolism in cells

- Messenger RNA controls the rate at which proteins are synthesized in cells.
- To reduce the amount of an enzyme a cell can decrease the rate of m-RNA transcription for its gene.
- Alternatively the cell can increase the rate at which the m-RNA is destroyed.

Protein Synthesis - Translation and Maturation

Genetic information flows from DNA to RNA to protein. DNA encodes the information required for synthesis of proteins and a copy of the encoded information is transcribed and processed into messenger RNA (mRNA). The information carried by the mRNA directs the synthesis of proteins, this process is called translation. Translation takes place on the surface of particles called ribosome's.

The Genetic Code

The genetic code is a system of specific base sequences that specify which amino acids are to be used for the synthesis of a protein during translation. The genetic code is comprised of codons which consist of a triplet of bases and specify a specific amino acid (Table 12.2). There are 64 codon sequences, sixty-one specify amino acids and three direct the termination of translation. Since there are 20 naturally occurring amino acids more than one codon can specify an amino acid. For example, isoleucine is specified by three codons ATT, ATC and ATA. Codons that specify the same amino acid are called synonyms. Synonymous codons generally differ in the third base, thus the genetic code is degenerate. The three codons, TAA, TAG and TGA are stop or nonsense codons and direct the termination of translation.

Table 12.2 : Chart showing codon sequences (Triplet codon) which specify specific Amino Acid.

First Base	Second Base				Third Base
	U	C	A	G	
U	Phenylalanine	Serine	Tyrosine	Cysteine	U
	Phenylalanine	Serine	Tyrosine	Cysteine	C
	Leucine	Serine	Stop (Ochre)	Stop (Opal)	A
	Leucine	Serine	Stop (Amber)	Tryptophan	G
C	Leucine	Proline	Histidine	Arginine	U
	Leucine	Proline	Histidine	Arginine	C
	Leucine	Proline	Glutamine	Arginine	A
	Leucine	Proline	Glutamine	Arginine	G
A	Isoleucine	Threonine	Asparagine	Serine	U
	Isoleucine	Threonine	Asparagine	Serine	C
	Isoleucine	Threonine	Lysine	Arginine	A
	Methionine (start)	Threonine	Lysine	Arginine	G
G	Valine	Alanine	Aspartic acid	Glycine	U
	Valine	Alanine	Aspartic acid	Glycine	C
	Valine	Alanine	Glutamic acid	Glycine	A

Translation is usually initiated with the codon AUG, which specifies Methionine. The remainder of the message is read sequentially, one codon at a time, the codons do not overlap, translation ceases when a stop codon is encountered.

For example, the DNA sequence 5' ATGGGTGGATATCCCTAG 3' is first transcribed into mRNA 5' AUGGGUGGAUAUCCCUAG 3' (notice the difference is Thymine becomes Uracil). This mRNA is then translated into Met-Gly-Gly-Tyr-Pro.

tRNA Structure and Function

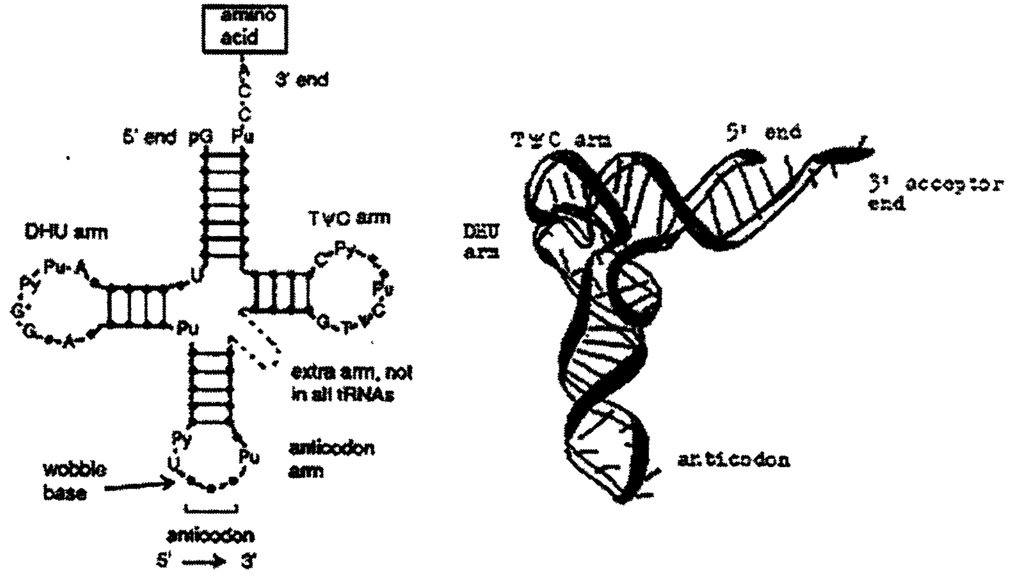
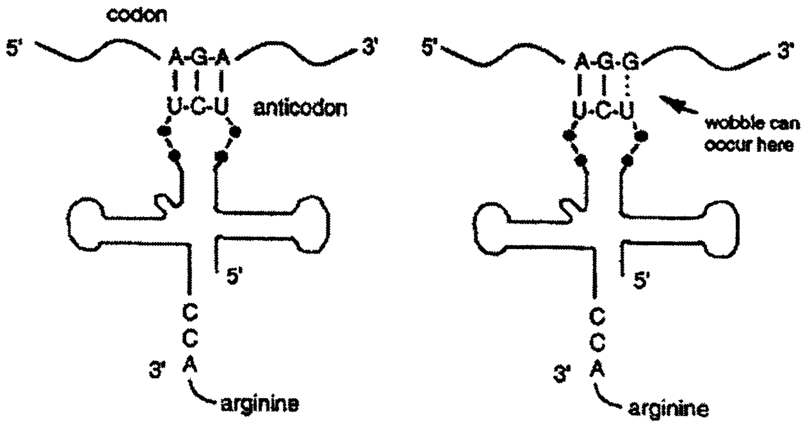

amino acid
3' end
A
C
C
5' end pG Pu
DHU arm
TΨC arm
Pu-A
Py
G
G
A
U
Pu
C
Py
Pu
C
G
T
Ψ
extra arm, not in all tRNAs
Py
U
Pu
wobble base
anticodon arm
anticodon
5' → 3'
TΨC arm
5' end
3' acceptor end
DHU arm
anticodon

Fig 12.5 : tRNA Structure

All tRNA's are similar in structure (Fig. 12.5). The T□C arm participates in binding of the charged tRNA to a site on the ribosome where protein synthesis occurs. The DHU (or D) arm is necessary for recognition by the proper aminoacyl tRNA synthase (the enzyme). The acceptor end is at the 3' terminus and ends in the sequence CAA. The anticodon arm consists of seven nucleotides, the sequence of which is read 3' to 5' (opposite convention to the usual 5' to 3'). The anticodon sequence is: 3' variable base'modified purine-X-Y-Z-Py-Py 5'. The central bases, X, Y, Z comprise the anticodon.


codon
5'
A-G-A
3'
U-C-U
anticodon
5'
C
C
3' A
arginine
5'
A-G-G
3'
U-C-U
wobble can occur here
5'
C
C
3' A
arginine

Each anticodon of a tRNA can base pair with a complementary codon on the tRNA. For example, Arginine is specified by two codons, AGA and AGG but there is only one tRNA anticodon for Arg, 3' UCU 5'. The tRNA recognizes and base pairs with either of the two Arg codons. Base pairing occurs between the first two bases of the codon and the anticodon, the third base of the codon does not match. Thus, base pairing is not strict for the last nucleotide of the codon anticodon pair, this phenomenon is called wobble.

Ribosome

Ribosome's are found in the cytoplasm, on the outer face of the rough ER and in the mitochondrial matrix. Ribosome's are composed of RNA and proteins. The sedimentation coefficient of eukaryotic ribosomes is 80S. The sedimentation coefficient, S, is a unit of measure that describes how fast a macromolecule will sediment when spun in a high speed centrifuge. Larger molecules generally have larger S values. Eukaryotic ribosomes consist of two subunits: a 60S large subunit and a 40S small subunit. The 60S subunit is comprised of about 45 proteins and three rRNA's that have S coefficients of 5, 5.8 and 28. The 40S subunit consists of about 33 proteins and 18S rRNA's.

Mitochondrial ribosomes are comprised of a large subunit, 16S rRNA and a small subunit 12S rRNA.

Protein Biosynthesis Process

Amino Acid Activation

Amino acids must be activated for translation to occur. Activation ensures that the correct amino acid will be recognized and that there is sufficient energy for peptide bond formation. Activation is the covalent coupling of amino acids to specific adapter molecules. The adapter molecules are called transfer RNA (tRNA). There is atleast one tRNA for each of the 20 naturally occurring amino acids. The tRNA recognize the codons carried by the mRNA and position them to facilitate peptide bond formation.

Step 1

Amino acid + ATP ----------> Amino-AMP-enz + PPi ; **enzyme**: Aminoacyl tRNA Synthase

The amino is linked via the 5' position to the ribose on the ATP, liberating PPi. Notice the amino-AMP complex remains bound to the enzyme, aminoacyl tRNA synthase.

Step 2

Amino-AMP-enz + tRNA ----------> amino-tRNA + AMP + Enzyme

The amino group is enzymatically transferred to the 3' terminal adenosine of tRNA, liberating the enzyme and AMP.

Over-all Equation:

Amino acid + ATP + tRNA ----------> Amino-tRNA + AMP + PPi

Note that two high energy phosphate bonds are used to form Amino-tRNA (Fig 12.6). A tRNA molecule that is linked to an amino acid is said to be charged.

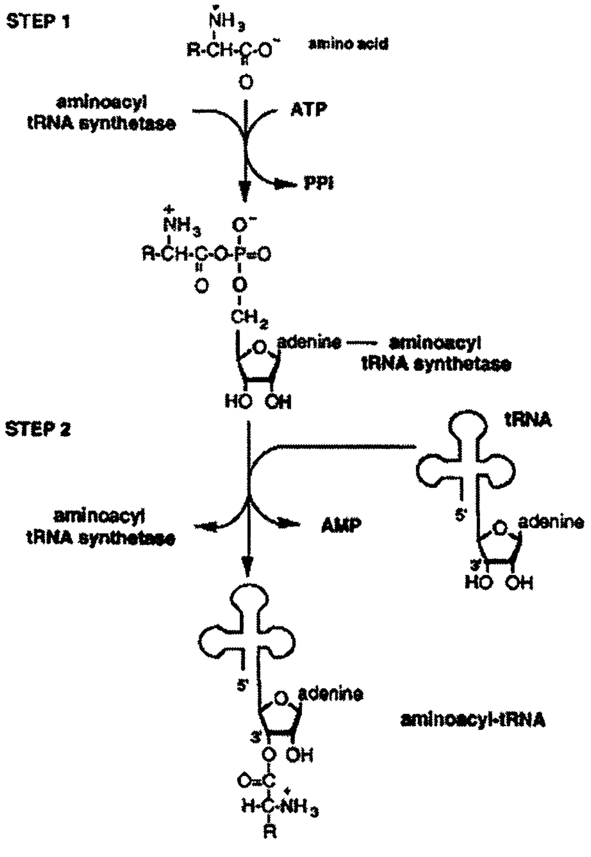

STEP 1
amino acid
aminoacyl tRNA synthetase
ATP
PPi
adenine — aminoacyl tRNA synthetase
HO OH
STEP 2
tRNA
5'
adenine
3'
HO OH
aminoacyl tRNA synthetase
AMP
5'
adenine
3'
aminoacyl-tRNA

Fig. 12.6 : Formation of Aminoacyl t-RNA

Translation of mRNA into a protein requires ribosomes, mRNA, tRNA, exogenous protein factors and energy in the form of ATP and GTP. Translation occurs in three major steps: initiation, elongation and termination (Table 12.3 and 12.4).

Initiation

Four major steps are required to initiate translation: ribosome dissociation, formation of a preinitiation complex, formation of the 40S initiation complex and formation of the 80S initiation complex.

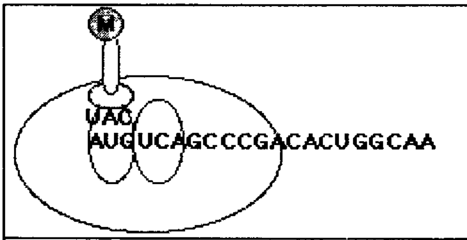
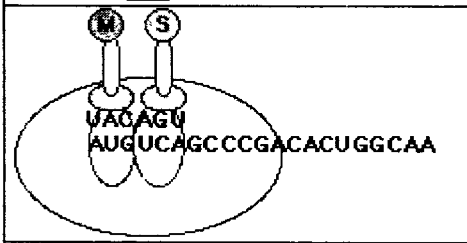
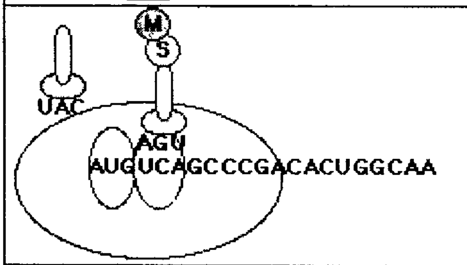
Elongation

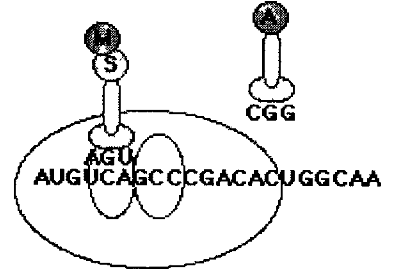
During elongation the protein is synthesized one amino acid at a time on the 80S ribosome. This process occurs in three major steps: binding of charged tRNA, peptide bond formation, translocation of the growing peptide chain.

Termination

When a stop codon appears at the A site translation is terminated. There are no tRNA's that recognize stop codons. Instead releasing factors, eRF, recognize the stop codon. The releasing factors along with peptidyl transferases and GTP catalyze the hydrolysis of the bond between the polypeptide chain and the tRNA. The protein and tRNA disassociate from the P site and the ribosome dissociates into the 40S and 60S subunits releasing the mRNA.

Table 12.3 : Major Steps in Protein Biosynthesis

	Initial steps: Messenger RNA is bound to ribosome with the start codon (AUG) at the P site. A transfer RNA molecule with the amino acid methionine (M) and the anticodon UAC has bound to the exposed start codon. The codon UCA is exposed at the A site.
	A second transfer RNA molecule, with the anticodon AGU and the amino acid serine (S) has bound to the A site.
	A peptide bond has formed between M and S and the peptide is bound to the A site. The methionine transfer RNA leaves, and the P site is exposed.



The ribosome has moved along the messenger RNA one codon, bringing the peptide to the P site. This exposes the A site and the next transfer RNA, carrying alanine (A) is about to bind.

Table 12.4 : Review of the Coding: DNA -> RNA -> Protein

	Start	1st Codon	2nd Codon	3rd Codon
DNA code	TAC	AGT	CGG	GCT
m-RNA code	AUG	UCA	GCC	CGA
t-RNA anticodon	UAC	AGU	CGG	GCU
Amino Acid	Methionine	Serine	Alanine	Arginine

Energy Cost for Protein Synthesis

- Charging of tRNA : 2 ATP's
- Binding of tRNA to Ribosome : 1 GTP
- Translocation : 1 GTP
- Total Cost 4 High Energy Phosphate Bonds for each Peptide Bond Formed

Protein Maturation

The rate of protein synthesis is about 6 peptide bonds per minute, thus, it takes, about 1 to 2 minutes to synthesize an average sized protein. Because mRNA is often several thousand nucleotides in length, the same mRNA molecules can be simultaneously bound by many ribosomes. An mRNA that is bound by multiple ribosomes is called a polysome. Polysomes provide a mechanism for many copies of a protein to be translated from a single mRNA. Polysomes in the cytosol synthesize most of the proteins and enzymes required by the body for intracellular processes such as metabolism.

When protein synthesis terminates, the initiator amino acid, Methionine, will have a free amino group. This end of the protein is the N terminus and the last amino acid of the chain has a free carboxy or C terminus. Protein synthesis thus initiates with the amino terminus and proceeds towards the C terminus. Proteins synthesized on the rough ER are transported across a membrane and into the cisternal spaces between the sheets of the ER where they are packaged for export.

To be transported across the membrane the protein is synthesized with a signal or leader sequence on its amino terminus.

After it is synthesized disulfide bonds are formed and the protein folds into its three dimensional state. Some proteins require post-translational modification before becoming fully active. These modifications can include removal of segments via peptidases, addition of phosphate, sugar or lipids to specific amino acids and glycosylation (Fig 12.7).

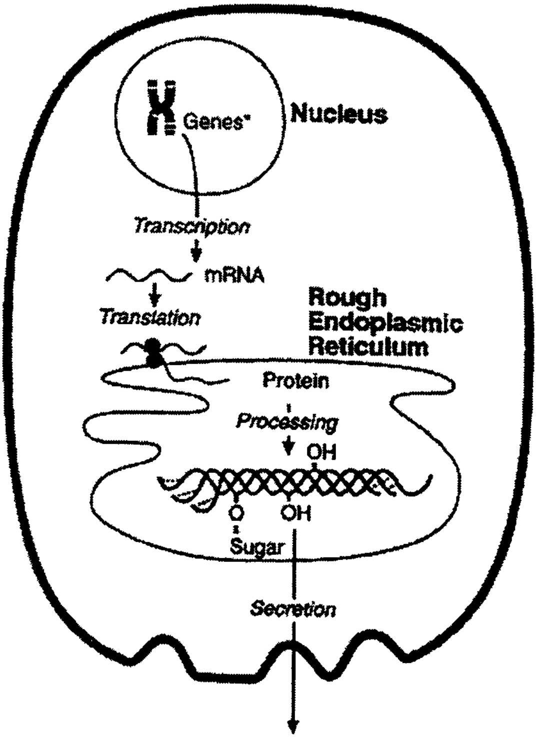

Genes*
Nucleus
Transcription
mRNA
Translation
Rough Endoplasmic Reticulum
Protein
Processing
OH
O
OH
Sugar
Secretion

Fig 12.7 : Overview of Protein biosynthesis and Post translational modifications.

Translational Inhibitors

Antibiotics

Streptomycin: prevents tRNA from binding, thus blocking the initiation of translation.

Erthyromycin: binds to the 50S subunit of the prokaryotic ribosome, blocking translocation.

Tetracycline: binds to the 30S subunit of the prokaryotic ribosome and inhibits binding of charged tRNA.

Toxins

Diptheria: catalyzes ADP ribosylation of a His in eEF inhibiting translocation. A few micrograms can kill a human being.

Ricin: cleaves a single adenine from 28S rRNA and inactivates the 60S ribosomal subunit. One molecule can kill an entire cell.

Protein Metabolism and Nitrogen Economy

A certain amount of dietary protein is required to synthesize endogenous proteins such as albumin (plasma protein), myosin(muscle filament), actin and hemoglobin. The basis for the dietary requirement of protein is the bodies inability to synthesize certain amino acids which is call essential amino cells (Table 12.5).

Table 12.5 : List of Essential and Non Essential Amino Acids

Essential	Non-Essential
Arginine[a]	Alanine
Histidine	Aspartic Acid
Isoleucine	Cysteine
Leucine	Glutamic Acid
Lysine	Glycine
Methionine[b]	Proline
Phenylalanine[c]	Serine
Threonine	Tyrosine
Tryptophan	Glutamine
Valine	Asparagine

[a] Arginine is synthesized by mammalian tissues, but the rate is not adequate during childhood.

[b] Methionine is required in large amounts to produce cysteine if the latter is dietarily lacking.

[c] Phenylalanine is needed in large amounts to produce tyrosine.

Protein Turnover:

- **Protein Balance**: interrelationship between protein synthesis and degradation (proteolysis).
- **Positive Nitrogen Balance**: when dietary intake of proteins is greater than the requirement for endogenous protein synthesis.

- **Neutral Nitrogen Balance**: dietary protein intake and endogenous proteins are maintained at a stable level.
- **Negative Nitrogen Balance**: if protein intake is insufficient or if the balance of amino acids is incorrect for synthetic needs, endogenous protein is metabolized to liberate free amino acids for synthesis of essential proteins.

Nitrogen Economy: (Fig. 12.8)

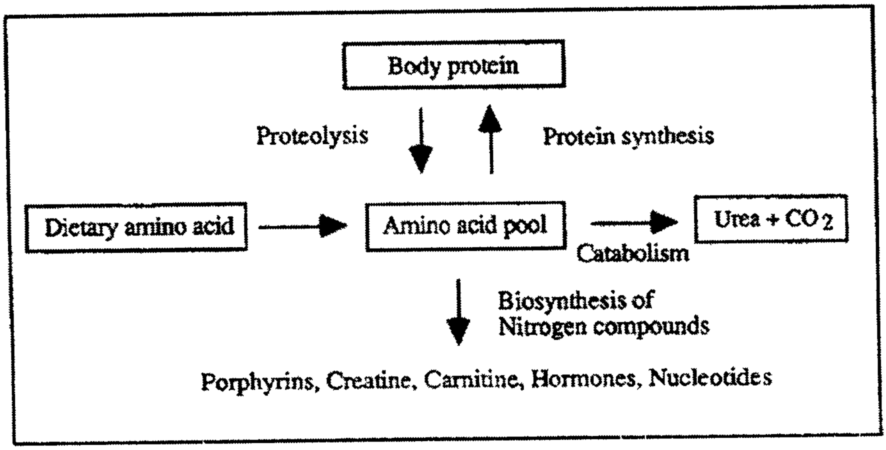

Body protein
Proteolysis
Protein synthesis
Dietary amino acid
Amino acid pool
Urea + CO2
Catabolism
Biosynthesis of Nitrogen compounds
Porphyrins, Creatine, Carnitine, Hormones, Nucleotides

Fig. 12.8 : Overview of Nitrogen Economy

Regulation of Protein Turnover and Nitrogen Economy:

- **Insulin**: increases synthesis, decreases degradation ----> favors maintenance of body protein pool.
- **Glucocorticoids**: released during stress or starvation, oppose the effects of insulin and result in protein degradation (metabolism).

Insulin / Glucocorticoid Ratio

Fed State (+ Insulin): High ratio ----> Protein Synthesis.

Fasting (- Insulin): Low ratio ----> Protein Degradation / Metabolism.

Roles of Protein Degradation / Metabolism:

- Digestion of dietary proteins.
- Activation of enzymes (zymogens).
- Fuel supply.

- Maintain amino acid pools.
- Control organ growth.
- Tissue repair.
- Removal of abnormal proteins.
- Complement and blood clotting cascades.

Some proteins degrade rapidly, $t_{1/2}$ = minutes, others slowly, $t_{1/2}$ = days.

Those that degrade rapidly may contain amino acid sequences which confer *instability*.

Others are "marked" by **ubiquitin**, a small protein forming a covalent linkage w/ proteins in an ATP dependent reaction.

QUIZ

Give Answer in brief

Q-1. In which form atmospheric nitrogen is used by all organisms?

A-1.

- Nitrogen ranks fourth important element after carbon, hydrogen and oxygen, of the mass of living cells.
- Though atmospheric nitrogen N_2 is most abundant but is too inert for use in most biochemical processes.
- Only few microorganisms can convert N_2 to biological useful form such as NH_3, amino groups are used with great economy in biological system.
- Atmospheric nitrogen needs to be converted by some organisms into forms acceptable to the all the organisms.
- Nitrogen fixation is carried out by bacterial *nitrogensases* forming reduced nitrogen, NH_4^+ that can then be used by all organisms to form amino acids.

Q-2. How nitrogen enters the human body?

A-2. Reduced nitrogen enters the human body as dietary free amino acids, protein, and the ammonia produced by intestinal tract bacteria. Amino acids derived from dietary proteins are the main source of amino groups.

Q-3. In which metabolic circumstances, amino acids in body can undergo oxidative degradation?

A-3. In body, amino acids can undergo oxidative degradation in three different metabolic conditions:

- During the normal synthesis and degradation of cellular proteins (protein turnover). Some of amino acids released during protein breakdown will undergo oxidative degradation if they are not needed for new protein synthesis.

- When a diet is rich in protein, the surplus amino acids may be catabolized when they are in excess. Amino acids cannot be stored.
- During starvation or in diabetes mellitus, when carbohydrates are either unavailable or not properly utilized, body proteins are used as fuel.
- Under these different circumstances amino acids lose their amino groups and the alpha-keto acids so formed may undergo oxidation to CO_2 and H_2O. In addition, the carbon skeleton of amino acids provides three and four carbon units that can be converted to glucose to be used by body.

Q-4. Does metabolic energy derived from amino acids varies greatly with the type of organism and with metabolic situation?

A-4.

- Carnivores immediately following a meal may obtain up to 90% of their energy requirements from amino acid oxidation.
- Herbivores may obtain a small fraction of their energy needs from amino acids.
- Most microorganisms can scavenge amino acids form their environment and can use for their metabolic need.
- Photosynthetic plants rarely oxidize amino acids for energy purpose. Instead they convert CO_2 and H_2O into carbohydrates that are mainly used as energy source.
- The amount of amino acids in plant tissues are carefully used for biosynthesis of proteins, nucleic acids and other molecules needed to support the growth.
- Amino acids catabolism does occur in plants and their metabolites are used for other biosynthetic pathways.

Q-5. What is gastrin and what is its role in protein digestion?

A-5. Entry of protein in to stomach stimulates gastric mucosa to secreate the hormone gastrin. Which stimulates the secretion of hydrochloric acid by the parietal cells of gastric glands and pepsinogen by the chief cells.

Q-6. How protein digestion takes place?

A-6. Protein digestion begins in the stomach, where a proenzyme called *pepsinogen* is secreted, autocatalytically converted to *Pepsin A*, and used for the first step of proteolysis. However, most proteolysis takes place in the duodenum as a consequence of enzyme activities secreted by the pancreas. All of the serine proteases and the zinc peptidases of pancreatic secretions are produced in the form of their respective proenzymes. These proteases are both endopeptidase and exopeptidase, and their combined action in the intestine leads to the production of amino acids, dipeptides, and tripeptides, all of which are taken up by enterocytes of the mucosal wall.

Q-7. How preoteolytic enzymes are regulated?

A-7. A circuitous regulatory pathway leading to the secretion of proenzymes into the intestine is triggered by the appearance of food in the intestinal lumen.

- Special mucosal endocrine cells secret the peptide hormones cholecystokinin (CCK) and secretin into the circulatory system.
- Together, CCK and secretin cause contraction of the gall bladder and the exocrine secretion of a bicarbonate-rich, alkaline fluid, containing protease proenzymes from the pancreas into the intestine.
- A second, paracrine role of CCK is to stimulate adjacent intestinal cells to secrete *enteropeptidase,* a protease that cleaves *trypsinogen* to produce *trypsin.*
- *Trypsin* also activates *trypsinogen* as well as all the other proenzymes in the pancreatic secretion, producing the active proteases and peptidases that hydrolyze dietary polypeptides.

Q-8. What is celiac disease?

A-8. Celiac disease is a condition in which the intestinal enzymes are unable to digest certain water insoluble proteins of wheat, particularly gliadin, which is injurious to the cells lining the small intestine. Wheat products must be avoided in this condition.

Q-9. What is acute pancreatitis?

A-9. In this condition the normal pathway of secretion of pancreatic juice into the intestine is obstructed. Thus the zymogens of the proteolytic enzymes are converted to the active forms inside the pancreatic cells, prematurely. This active proteolytic enzymes act on the pancreatic tissue itself, causing serious destruction of pancreas, which is very painful and can be fatal.

Q-10. How intestinal bacterial activity contributes to nitrogen metabolism?

A-10. Many other nitrogenous compounds are formed in the intestine as a result of intestinal bacterial activity. Some have powerful pharmacological (vasopressor) effects. Intestinal bacteria convert lysine, arginine, tyrosine, ornithine and histidine to their vasopressor amines such as cadaverene, agmatine, tyramine, putrescine and histamine respectively.

Q-11. What are essential amino acids?

A-11. Prokaryotes such as *E. coli* can make the carbon skeletons of all 20 amino acids and transaminate those carbon skeletons with nitrogen from glutamine or glutamate to complete the amino acid structures.

Humans cannot synthesize the branched carbon chains found in branched chain amino acids or the ring systems found in phenylalanine and the aromatic amino acids; nor can we incorporate sulfur into covalently bonded structures.

Therefore, the 8 so-called essential amino acids must be supplied from the diet. They are:

Branched chain amino acids: leucine, Isoleucine, Valine,
Aromatic amino acids: Phenylalanine, Tryptophan,
Sulphur containing amino acid: Methionine
Basic amino acids: Lysine and Threonine.

Q-12. What are semi-essential amino acids?

A-12. Histidine and arginine are not usually considered to be essential, because enough for adult needs is made by the urea cycle. However, the urea cycle generally does not provide sufficient arginine for the needs of a growing child.

Q-13. What happens during the degradation of amino acids?

A-13. Amino acids can undergo oxidative degradation as a consequence of protein turnover; when the diet is particularly rich in protein or when carbohydrates are not available like in starvation or in diabetes mellitus.

The degradative pathway of every amino acid requires the separation of the amino group from the carbon skeleton. The carbon skeletons enter the Krebs cycle or are channeled into gluconeogenesis. Part of the ammonia is reused for biosynthetic purpose; part is excreted directly and the rest is excreted as urea.

Q-14. How removal of nitrogen from amino acids takes place?

A-14. Most of the amino acids are metabolized in liver. Some of the ammonia that is generated is recycled and used in a variety of biosynthetic processes. The excess ammonia is either excreted directly or converted to uric acid or urea for excretion depending on the organism. Excess ammonia generated in extrahepatic tissues is transported to the liver for excretion after converting to a proper form. Nitrogen elimination begins intracellularly with protein degradation. There are two main routes for converting intracellular proteins to free amino acids: a lysosomal pathway, by which extracellular and some intracellular proteins are degraded, and cytosolic pathways that are important in degrading proteins of intracellular origin.

Q-15. What happens in cystosolic pathway?

Q-15. In one cytosolic pathway:

- A protein known as ubiquitin is activated by conversion to an AMP derivative.
- And cytosolic proteins that are damaged or otherwise destined for degradation are enzymically tagged with the activated ubiquitin.
- Ubiquitin-tagged proteins are then attacked by cytosolic ATP-dependent proteases that hydrolyze the targeted protein, releasing the ubiquitin for further rounds of protein targeting.

Q-16. How amino acid nitrogen is removed?

A-16. The dominant reactions involved in removing amino acid nitrogen from the body are known as transaminations. This class of reactions funnels nitrogen from all free amino acids into a small number of compounds; then, either they are oxidatively deaminated, producing ammonia, or their amine groups are converted to urea by the urea cycle.

Q-17. How transaminations take place?

A-17. Transaminations involve moving a α-amino group from a donor α-amino acid to the keto carbon of an acceptor α-keto acid. These reversible reactions are catalyzed by a group of intracellular enzymes known as transaminases (aminotransferases), which employ covalently bound pyridoxal phosphate as a cofactor.

Transaminases exist for all amino acids except threonine and lysine.

Q-18. Which compounds are most commonly involved in transamination?

A-18. The most common compounds involved as a donor/acceptor pair in transamination reactions are glutamic acid and α-ketoglutaric acid, which participate in reactions with many different aminotransferases.

Q-19. Which aminotransferase is clinically important?

A-19. Serum aminotransferases such as *serum glutamate-oxaloacetate-aminotransferase* (SGOT) have been used as clinical markers of tissue damage, with increasing serum levels indicating an increased extent of damage.

Q-20. How creatinine is formed and what is its clinical significance?

A-20.

- The first reaction in creatinine formation is the transfer of the amido (or amidine) group of arginine to glycine, forming guanidinoacetate.
- Subsequently, a methyl group is transferred from the ubiquitous 1-carbon-donor S-adenosylmethionine to guanidinoacetate to produce creatine (from which phosphocreatine is formed), some of which spontaneously cyclizes to creatinine, and is eliminated in the urine.
- The quantity of urine creatinine is generally constant for an individual and approximately proportional to muscle mass.
- In individuals with damaged muscle cells, creatine leaks out of the damaged tissue and is rapidly cyclized, greatly increasing the quantity of circulating and urinary creatinine.
- A small but clinically important amount of creatinine is excreted in the urine daily, and the creatinine clearance rate is often used as an indicator of kidney function.

Q-21. How glutamate is a prominent intermediate in nitrogen elimination?

A-21.

- Because of the participation of α-ketoglutarate in numerous transaminations, glutamate is a prominent intermediate in nitrogen elimination as well as in anabolic pathways.
- Glutamate formed in the course of nitrogen elimination is either oxidatively deaminated by liver *glutamate dehydrogenase,* forming ammonia.
- The ammonia thus formed is converted to glutamine by *glutamine synthase* and transported to kidney tubule cells.
- There the glutamine is sequentially deamidated by *glutaminase* and deaminated by kidney *glutamate dehydrogenase.*
- The ammonia produced in the latter two reactions is excreted as NH_4^+ in the urine, where it helps maintain urine pH in the normal range of pH 4 to pH 8.
- The extensive production of ammonia by peripheral or liver *glutamate dehydrogenase* is not feasible because of the highly toxic effects of circulating ammonia.
- Normal serum ammonium concentrations are in the range of 20-40 mmol, and an increase in circulating ammonia to about 400 mmol causes alkalosis and neurotoxicity.

Q-22. Which amino acid related reaction is therapeutically significant?

A-22.

- A therapeutically useful amino acid-related reaction is the amidation of aspartic acid to produce asparagine.
- The enzyme *asparagine synthase* catalyzes the ATP, requiring the transamidation reaction shown below: aspartate + glutamine + ATP --> glutamate + asparagine + AMP + PP_i
- Most cells perform this reaction well enough to produce all the asparagine they need.
- However, some leukemia cells require exogenous asparagine, which they obtain from the plasma. Chemotherapy using the enzyme *asparaginase* takes advantage of this property of leukemic cells by hydrolyzing serum asparagine to ammonia and aspartic acid, thus depriving the neoplastic cells of the asparagine that is essential for their characteristic rapid growth.

Q-23. How clinically important are sterospecific amino acid oxidases?

A-23.

- In the peroxisomes of mammalian tissues, especially liver, there are 2 stereospecific *amino acid oxidases* involved in elimination of amino acid nitrogen.

- *D-amino acid oxidase* is an FAD-linked enzyme, and while there are few D-amino acids that enter the human body the activity of this enzyme in liver is quite high. *L-amino acid oxidase* is FMN-linked and has broad specificity for the L amino acids.
- A number of substances, including oxygen, can act as electron acceptors from the flavoproteins. If oxygen is the acceptor the product is hydrogen peroxide, which is then rapidly degraded by the *catalases* found in liver and other tissues.
- Missing or defective biogenesis of peroxisomes or *L-amino acid oxidase* causes generalized hyper-aminoacidemia and hyper-aminoaciduria, generally leading to neurotoxicity and early death.

Q-24. Describe the role and significance of glutamate dehydrogenase?

A-24.

The reaction catalyzed by *glutamate dehydrogenase* is:

NH_4^+ + α -ketoglutarate + NAD (P) H + H^+ <----> glutamate + NAD $(P)^+$ + H_2O

Glutamate dehydrogenase can utilize either NAD orNADP as cofactor.

- In the forward reaction as shown above *glutamate dehydrogenase* is important in converting free ammonia and α-ketoglutarate (α-KG) to glutamate, forming one of the 20 amino acids required for protein synthesis.
- However, it should be recognized that the reverse reaction is a key anapleurotic process linking amino acid metabolism with TCA cycle activity.
- In the backward reaction, *glutamate dehydrogenase* provides an oxidizable carbon source used for the production of energy.
- As expected for a branch point enzyme with an important link to energy metabolism, *glutamate dehydrogenase* is regulated by the cell energy charge.
- ATP and GTP are negative effectors, whereas ADP and GDP are positive allosteric effectors. Thus, when the level of ATP is high, conversion of glutamate to α-KG and other TCA cycle intermediates is limited; when the cellular energy charge is low, glutamate is converted to ammonia and oxidizable TCA cycle intermediates.

Q-25. Describe the role and significance of glutamine synthase.

A-25.

The reaction catalyzed by *glutamine synthase* is:

glutamate + NH_4^+ + ATP -------> glutamine + ADP + P_i + H^+

The *glutamine synthatase* reaction is also important in several respects. First it produces glutamine, one of the 20 major amino acids. Second, in animals, glutamine is the major amino acid found in the circulatory system.

Its role there is to carry ammonia to and from various tissues but principally from peripheral tissues to the kidney, where the amide nitrogen is hydrolyzed by the enzyme *glutaminase* (reaction below); this process regenerates glutamate and free ammonium ion, which is excreted in the urine.

$$\text{glutamine} + H_2O \text{ -------> } \text{glutamate} + NH_3$$

Q26. What is Urea Cycle?

A26. About 80% of the excreted nitrogen is in the form of urea, which is also largely made in the liver, in a series of reactions that are distributed between the mitochondrial matrix and the cytosol. The series of reactions that form urea is known as the Urea Cycle or the Krebs-Henseleit Cycle.

Q-27. What are essential features of the urea cycle?

A-27.

- Arginine from the diet or from protein breakdown is cleaved by the cytosolic enzyme *arginase,* generating urea and ornithine.
- Ornithine arising in the cytosol is transported to the mitochondrial matrix, where *ornithine transcabamoylase* catalyzes the condensation of ornithine with carbamoyl phosphate, producing citrulline. The energy for the reaction is provided by the high-energy anhydride of carbamoyl phosphate.
- The product, citrulline, is then transported to the cytosol, where the remaining reactions of the cycle take place.
- In a 2-step reaction, catalyzed by cytosolic *argininosuccinate synthetase,* citrulline is converted to argininosuccinate. The reaction involves the addition of AMP (from ATP) to the amido carbonyl of citrulline, forming an activated intermediate on the enzyme surface (AMP-citrulline), and the subsequent addition of aspartate to form argininosuccinate.
- Arginine and fumarate are produced from argininosuccinate by the cytosolic enzyme *argininosuccinate lyase.* In the final step of the cycle *arginase* cleaves urea from aspartate, regenerating cytosolic ornithine, which can be transported to the mitochondrial matrix for another round of urea synthesis.
- Beginning and ending with ornithine, the reactions of the cycle consumes 3 equivalents of ATP and a total of 4 high-energy nucleotide phosphates. Urea is the only new compound generated by the cycle; all other intermediates and reactants are recycled.

Q-28. How the regulation of the Urea Cycle takes place in the body?

A-28. The urea cycle operates only to eliminate excess nitrogen. On high-protein diets the carbon skeletons of the amino acids are oxidized for energy or stored as fat and glycogen, but the amino nitrogen must be excreted.

To facilitate this process, enzymes of the urea cycle are controlled at the gene level. When dietary proteins increase significantly, enzyme concentrations rise. On return to a balanced diet, enzyme levels decline. Under conditions of starvation, enzyme levels rise as proteins are degraded and amino acid carbon skeletons are used to provide energy, thus increasing the quantity of nitrogen that must be excreted.

Q-29. What happens when excretion of ammonia is deranged?

A-29. Built up of ammonia is neurotoxic. Marked brain damage is seen in cases of failure to make urea via the urea cycle or to eliminate urea through the kidneys. The result of either of these events is a buildup of circulating levels of ammonium ion. Aside from its effect on blood pH, ammonia readily traverses the brain blood barrier and in the brain is converted to glutamate via *glutamate dehydrogenase,* depleting the brain of α-ketoglutarate. As the α-ketoglutarate is depleted oxaloacetate falls correspondingly, and ultimately TCA cycle activity comes to a halt. In the absence of aerobic oxidative phosphorylation and TCA cycle activity, irreparable cell damage and neural cell death ensue.

Q-30. What are the uses of glycine ?

A-30. Glycine is involved in the synthesis of haemoglobin, creatine phosphate, purines, glutathione, proteins, phospholipids, bile acids and detoxification reactions.

Identify the letter of the choice that best completes the statement or Answers the question.

1. The significance of 64 possible codons is:
   A. The majority of codons are nonsense and do not code for any amino acid.
   B. The code can overlap yet always encode an amino acid.
   C. The majority of codons code for some amino acid, since each amino acid can be coded by several triplets.
   D. Commas are present to indicate the appropriate grouping of triplets.

Answer: C

2. Which of the following codons are termination codons?
   A. UUC B. UUA C. UAG D. UAC

Answer: C

3. How does accurate translation occur by aminoacyl tRNAs?
   A. The activated amino acid part of aminoacyl tRNA interacts only with its respective codon.

B. The anticodon loop of aminoacyl tRNA base pairs with its corresponding codon.
C. Specific aminoacyl tRNA synthetases recognize specific identity elements of tRNA and bind the appropriate amino acid to the tRNA.
D. B and C

Answer: D

4. All of the following statements concerning aminoacyl tRNA synthetase are true except:
A. The hydrolysis of ATP to ADP makes the enzymatic reaction thermodynamically favorable.
B. Aminoacyl tRNA synthetase converts ATP to AMP and pyrophosphate.
C. Pyrophospate produced by aminoacyl tRNA synthetase is hydrolyzed and makes the reaction irreversible.
D. Aminoacyl tRNA synthetases form an enzyme bound aminoacyl adenylate intermediate.

Answer: A

5. One of the codons for serine is 5'-AGC-3". The anticodon is:
A. 5'-UCG-3' B. 5'-GCU-3' C. 5'-TCG-3' D. 5'-GCT-3'

Answer: B

6. Inosine is a base used in anticodons that base pairs with:
A. U B. C C. U,C,A D. A

Answer: C

7. In which of the following compartments of a mammalian cell can protein synthesis occur?
A. lysosome B. Golgi apparatus
C. mitochondria D. endoplasmic reticulum

Answer: C

8. In the centrifugal separation of an eukaryotic cell, you might expect to detect ribosomes with what sedimentation coefficient?
A. 80S B. 60S C. 80S and 70S D. 80S and 60S

Answer: C

9. In prokaryotic translation, where the initiation codon interacts with the anticodon of f-Met-tRNAfmet, what is the sequence of the anticodon?
A. 3'-AUG-5' B. 3'-UAC-5'
C. 5'-CAU-3' D. 5'-CAU-3'

Answer: D

10. During elongation, as mRNA moves through the ribosome in association with the anticodons of tRNA, which site is occupied by the growing protein attached to a tRNA?
A. A-site B. P-site C. E-site D. L-site

Answer: B

11. The energy required for the formation of a new peptide bond (transpeptidation) is supplied by:
A. ATP B. GTP C. pyrophosphate D. none

Answer: D

12. Energy for the movement of peptidyl-tRNA from the A-site to the P-site is provided by:
A. ATP B. GTP C. pyrophosphate D. none

Answer: B

13. Predict the energy cost for biosynthesis of a 100 residue protein.
A. 200 phosphoric anhydride bonds
B. 100 phosphoric anhydride bonds
C. 99 phosphoric anhydride bonds
D. 396 phosphoric anhydride bonds

Answer: D

14. Termination of protein synthesis occurs when the polypeptidyl chain is transferred to a water molecule rather than an aminoacyl-tRNA. What molecule provides energy for this transfer?
A. ATP B. GTP
C. pyrophosphate D. none of the above

Answer: D

15. Which of the following is not found in eukaryotic mRNA?
A. AUG-start codon
B. Shine-Delgarno sequence
C. 5'-7methyl-GTP cap
D. poly (A) tail

Answer: B

Complete each sentence or statement.

1. ________ is the transfer of an intermediate product from one enzyme active site to another in such a way that the intermediate remains in contact with the protein.

Answer: Channeling

2. The nitrogen cycle is a set of reactions, including nitrogen fixation, ________, and denitrification, for the interconversion of different forms of nitrogen.

Answer: nitrification

3. A cyclic metabolic pathway in which amino groups are converted to urea for disposal is the ________.

Answer: urea cycle

4. A/An ________ is an amino acid that an animal cannot synthesize.

Answer: essential amino acid

5. A/An ________ reincorporates an intermediate of nucleotide degradation into a new nucleotide, thereby minimizing the need for the nucleotide biosynthetic pathways.

Answer: salvage reaction

6. A/An ________ amino acid can be degraded to a gluconeogenic precursor.

Answer: glucogenic

7. A/An ________ is a substance released by a nerve cell to alter the activity of a target cell.

Answer: neurotransmitter

8. An amino acid whose degradation yields compounds that can be converted to fatty acids or ketone bodies but not to glucose is a/an ________.

Answer: ketogenic amino acid

9. An amino acid that an organism can synthesize from common intermediates is a/an ________.

Answer: non essential amino acid

10. The conversion of atmospheric nitrogen to a biologically useful form is known as ________.

Answer: nitrogen fixation

11. ________ functions as a cofactor in the transfer of one-carbon groups.

Answer: Tetrahydrofolate

12. ________ catalyzes the conversion of ribonucleoside diphosphates to deoxyribonucleoside diphosphates.

Answer: Ribonucleotides reductase

13. Due to their role in nitrogen metabolism, the most abundant amino acids in mammals are glutamate and ________.

Answer: glutamine

14. Pyruvate can be converted to the amino acid ________ in one step.

Answer: alanine

15. Aspartate can be converted to the citric acid cycle intermediate ________ in one step.

Answer: oxaloacetate

16. A/An ________ catalyzes the transfer of an amino group to an a-keto acid.

Answer: transaminase

17. The ________ is the ribosomal binding site that accommodates an aminoacyl-tRNA.

Answer: A site

18. ________ is the complex of membrane proteins that mediates the transmembrane movement of a polypeptide.

Answer: Translocon

19. A code in which more than one "word" encodes the same entity is said to be ________.

Answer: degenerate

20. Release factor (RF) is a protein that recognizes a stop ________ and causes a ribosome to terminate polypeptide synthesis.

Answer: codon

21. Signal peptide is a short sequence in a membrane or secretory protein that binds to the ________ in order to direct the translocation of the protein across a membrane.

Answer: signal recognition particle

22. The sequence of three nucleotides in a tRNA that recognizes an mRNA codon through complementary base pairing is a/an ________.

Answer: anticodon

23. The ________ is an explanation for the nonstandard base pairing between tRNA and mRNA at the third codon position, which allows a tRNA to recognize more than one codon.

Answer: Wobble hypothesis

24. The ribosomal binding site that accommodates a peptidyl-tRNA is the ________.

Answer: P site

25. ________ is an mRNA transcript bearing multiple ribosomes in the process of translating the mRNA.

Answer: Polysome or Polyribosome

26. A protein that interacts with mRNA and/or the ribosome and which is required to initiate translation is a/an ________.

Answer: initiation factor (IF)

27. A tRNA that carries the same amino acid as another tRNA but has a different codon is a/an ________.

Answer: isoacceptor tRNA

28. ________ is the ribosomal process in which the peptidyl group attached to a tRNA is transferred to the aminoacyl group of another tRNA forming a new peptide bond and lengthening the polypeptide by one residue at its C-terminus.

Answer: Transpeptidation

29. The grouping of nucleotides in sets of three whose sequence corresponds to a polypeptide sequence is the ________.

Answer: reading frame

30. ________ is the attachment of carbohydrate chains to a protein through N- or O-glycosidic linkages.

Answer: Glycosylation

31. The process of transforming the information contained in the nucleotide sequence of an RNA to the corresponding amino acid sequence of a polypeptide as specified by the genetic code is called ________.

Answer: translation

32. A ribosome is the complex of RNA and protein that synthesizes polypeptides under the direction of ________.

Answer: mRNA

33. ________ is a mechanism for promoting translational accuracy in which a noncognate tRNA dissociates from the ribosome before EF-Tu hydrolyzes its GTP.

Answer: Kinetic proofreading

34. A protein that binds to a ribosome after protein synthesis to prepare it for another round of translation is a/an ________.

Answer: ribosome recycling factor (RRF)

35. The ribosomal binding site that accommodates a deacylated-tRNA before it dissociates from the ribosome is the ________.

Answer: E site

36. ________ is the movement of tRNA and mRNA, relative to the ribosome, that occurs following formation of a peptide bond and that allows the next mRNA codon to be translated.

Answer: Translocation

37. In eukaryotes, gene expression is related to the coiling and uncoiling of ____________________.

Answer: DNA

38. A DNA subunit composed of a phosphate group, a five-carbon sugar, and a nitrogen-containing base is called a(n) ____________________.

Answer: nucleotide

39. The name of the five-carbon sugar that makes up a part of the backbone of molecules of DNA is ____________________.

Answer: deoxyribose

40. Knowing the order of the bases in a gene permits scientists to determine the exact order of the amino acids in the expressed ____________________.

Answer: protein

41. Due to the strict pairing of nitrogen base pairs in DNA molecules, the two strands are said to be ____________________ to each other.

Answer: complementary

42. According to base-pairing rules, adenine pairs with ____________________ and guanine pairs with ____________________.

Answer: thymine, cytosine

43. The enzyme that is responsible for replicating molecules of DNA by attaching complementary bases in the correct sequence is ____________________.

Answer: DNA polymerase

44. Enzymes called ____________________ are responsible for unwinding the DNA double helix by breaking the hydrogen bonds that hold the complementary strands together.

Answer: helicases

45. The process by which DNA copies itself is called ____________________.

Answer: replication

46. Molecules of ____________________ carry instructions for protein synthesis from the nucleus to the cytoplasm.

Answer: RNA

47. The nitrogen-containing base that is found only in RNA is ____________________.

Answer: uracil

48. The enzyme responsible for making RNA is called ____________________.

Answer: RNA polymerase

49. The form of ribonucleic acid that carries genetic information from the DNA to the ribosomes is ____________________.

Answer: mRNA

50. A ____________________ is a sequence of DNA at the beginning of a gene that signals RNA polymerase to begin transcription.

Answer: promoter

51. Messenger RNA is produced during the process of ____________________.

Answer: transcription

52. Of the 64 codons of mRNA, 61 code for ____________________, 3 are ____________________ signals, and one is a ____________________ signal.

Answer: amino acids; stop; start

53. Nucleotide sequences of tRNA that are complementary to codons on mRNA are called ____________________.

Answer: anticodons

54. The sequence of three nucleotides that code for specific amino acids or stop signals in the synthesis of protein is called a(n) ____________________.

Answer: codon

55. The information contained in a molecule of messenger RNA is used to make protein during the process of ____________________.

Answer: translation

56. During translation, amino acids are brought to the ribosomes by molecules of ____________________.

Answer: transfer RNA

Chapter 13

Photosynthesis

Photosynthesis is the process of converting light energy to chemical energy and storing it in the bonds of sugar. This process occurs in plants and some algae (Kingdom Protista). Plants need only light energy, CO_2, and H_2O to make sugar. The process of photosynthesis takes place in the chloroplasts, (Fig. 13.1) specifically using chlorophyll, (Fig. 13.2) the green pigment involved in photosynthesis.

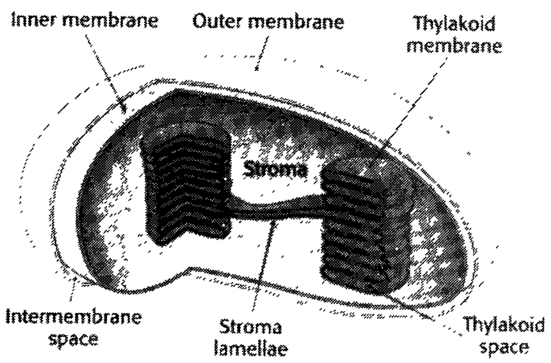

Inner membrane
Outer membrane
Thylakoid membrane
Stroma
Intermembrane space
Stroma lamellae
Thylakoid space

Fig. 13.1 : The Chloroplast

Photosynthesis takes place primarily in plant leaves, and little to none occurs in stems, etc. The parts of a typical leaf include the **upper and lower** epidermis, the mesophyll, the **vascular bundle(s)** (veins), and the stomates. The upper and lower epidermal cells do not have chloroplasts, thus photosynthesis does not occur there. They serve primarily as protection for the rest of the leaf. The stomates are holes which occur primarily in the lower epidermis and are for air exchange: they let CO_2 in and O_2 out. The vascular bundles or veins in a leaf are part of the plant's transportation system, moving water and nutrients around the plant

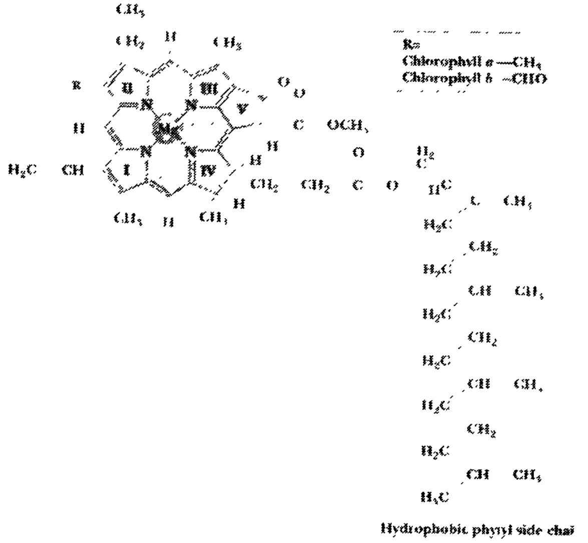

R=
Chlorophyll a —CH3
Chlorophyll b —CHO
Mg
Hydrophobic phytyl side chain

Fig. 13.2 : Structure of Chlorophyll a and b

as needed. The mesophyll cells have chloroplasts and this is where photosynthesis occurs.

As you hopefully recall, the parts of a chloroplast include the outer and inner membranes, intermembrane space, stroma, and thylakoids stacked in grana. The chlorophyll is built into the membranes of the thylakoids.

Chlorophyll looks green because it absorbs red and blue light, making these colours unavailable to be seen by our eyes. It is the green light which is NOT absorbed that finally reaches our eyes, making chlorophyll appear green. However, it is the energy from the red and blue light that are absorbed that is, thereby, able to be used to do photosynthesis. The green light we can see is not/cannot be absorbed by the plant, and thus cannot be used to do photosynthesis.

The overall chemical reaction involved in photosynthesis is:

$$\boxed{6H_2O + 6CO_2 \rightarrow \text{Glucose} + 6O_2}$$

There are two parts to photosynthesis:

The **light reaction** happens in the thylakoid membrane and converts light energy to chemical energy. This chemical reaction must, therefore, take place in the light. Chlorophyll and several other pigments such as **beta-carotene** are organized in clusters in the thylakoid membrane and are involved in the light reaction. Each of these differently-coloured pigments can absorb a slightly different colour of light and pass its energy to the central chlorphyll molecule to do photosynthesis. The central part of the chemical structure of a chlorophyll molecule is a porphyrin ring, which consists of several fused rings of carbon and nitrogen with a magnesium ion in the center.

The energy harvested via the light reaction is stored by forming a chemical called ATP (adenosine triphosphate), a compound used by cells for energy storage. This chemical is made of the nucleotide adenine bonded to a ribose sugar, and that is bonded to three phosphate groups. This molecule is very similar to the building blocks for our DNA.

The **dark reaction** takes place in the stroma within the chloroplast, and converts CO_2 to sugar. This reaction doesn't directly need light in order to occur, but it does need the products of the light reaction (ATP and another chemical called NADPH). The dark reaction involves a cycle called the **Calvin cycle** in which CO_2 and energy from ATP are used to form sugar. Actually, notice that the first product of photosynthesis is a three-carbon compound called glyceraldehyde 3-phosphate. Almost immediately, two of these join to form a glucose molecule.

Most plants put CO_2 directly into the Calvin cycle. Thus the first stable organic compound formed is the glyceraldehyde 3-phosphate. Since that molecule contains three carbon atoms, these plants are called **C_3 plants**. For all plants, hot summer weather increases the amount of water that evaporates from the plant. Plants lessen the amount of water that evaporates by keeping their stomates closed during hot, dry weather. Unfortunately, this means that once the CO_2 in their leaves reaches a low level, they must stop doing photosynthesis. Even if there is a tiny bit of CO_2 left, the enzymes used to grab it and put it into the Calvin cycle just don't have enough CO_2 to use. Typically the grass in our yards just turns brown and goes dormant. Some plants like **crabgrass**, **corn**, and **sugar cane** have a special modification to conserve water. These plants capture CO_2 in a different way: they do an extra step first, before doing the Calvin cycle. These plants have a special enzyme that can work better, even at very low CO_2 levels, to grab CO_2 and turn it first into oxaloacetate, which contains four carbons. Thus, these plants are called **C_4 plants**. The CO_2 is then released from the oxaloacetate and put into the Calvin cycle. This is why crabgrass can stay green and keep growing when all the rest of your grass is dried up and brown.

There is yet another strategy to cope with very hot, dry, desert weather and conserve water. Some plants (for example, cacti and pineapple) that live in extremely hot, dry areas like deserts, can only safely open their stomates at night when the weather is cool. Thus, there is no chance for them to get the CO_2 needed for the dark reaction during the daytime. At night when they can open their stomates and take in CO_2, these plants incorporate the CO_2 into various organic compounds to store it. In the daytime, when the light reaction is occurring and ATP is available (but the stomates must remain closed), they take the CO_2 from these organic compounds and put it into the Calvin cycle. These plants are called **CAM plants**, which stands for crassulacean acid metabolism after the plant family, Crassulaceae (which includes the garden plant *Sedum*) where this process was first discovered.

Photosynthesis has two sets of reactions: (Fig. 13.3)

- Light reactions split water,
    - Hydrogens are used to produce a reduced coenzyme, NADPH, and ATP.
    - Oxygen is given off.
- Once the NADPH and ATP are formed the rest of the reactions can take place in the dark.

- CO2 is reduced to glucose.
- A set of cyclic reactions: the Calvin cycle.

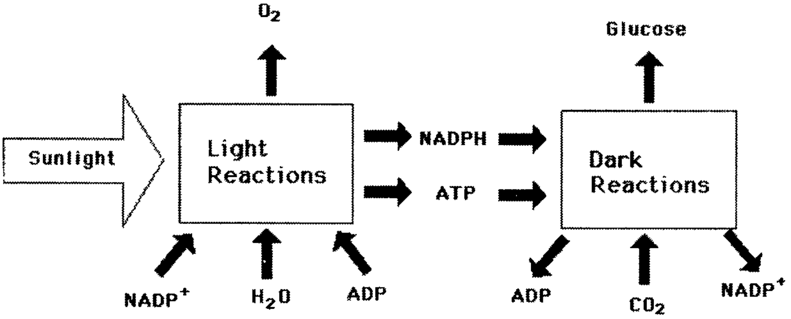

O_2
Glucose
Sunlight
Light Reactions
NADPH
ATP
Dark Reactions
$NADP^+$
H_2O
ADP
ADP
CO_2
$NADP^+$

Fig. 13.3 : Two sets & Reactions during photosynthesis.

In land plants and green algae (chlorophytes), photosynthesis has two distinct stages, the light reactions, which convert light energy to ATP and NADPH; and the dark reactions, which convert CO_2 to carbohydrate using ATP and NADPH. Both occur in the chloroplasts (Fig. 13.4).

Light Reactions:

$$12H_2O + 12NADP + 18ADP \longrightarrow 6O_2 + 12NADPH + 18ATP$$

Dark Reactions

$$6CO_2 + 12NADPH + 18ATP \longrightarrow C_6H_{12}O_6 + 12NADP + 18ADP + 6H_2O$$

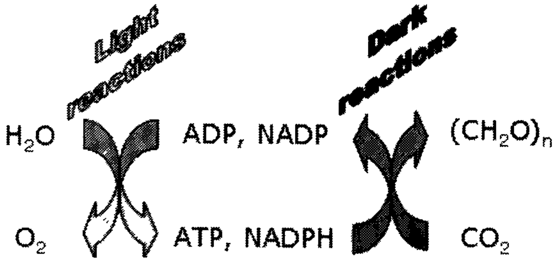

Light reactions
Dark reactions
H_2O
ADP, NADP
$(CH_2O)_n$
O_2
ATP, NADPH
CO_2

Fig. 13.4 : Link between Light and Dark Reaction

Light Reaction of Photosynthesis

The light (dependent) reactions take place on chloroplast membranes and generate ATP and NADPH. These cofactors are mostly used for the reduction of CO_2 to carbohydrates, but some ATP and NADPH can be used for other metabolic processes. They only occur when light is present.

In green plants and other algae, photosynthesis goes on in the chloroplasts. These probably evolved by endosymbiosis. Some chloroplasts are probably secondary endosymbionts. As for mitochondria, the evidence is extensive.

- Chloroplasts have an outer (host) membrane and an inner (bacterial) membrane.
- The inner membrane is folded inwards to form sacs called lamellae. The membranes these lamellae are made of are called thylakoid membranes, which look just like those of cyanobacteria (blue-green algae) (Fig 13.5).

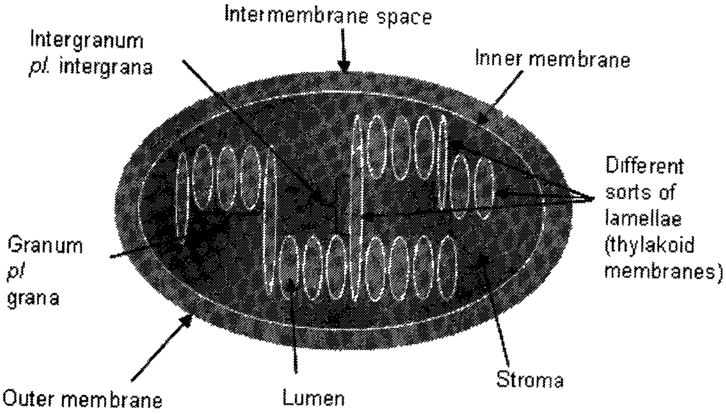


Fig. 13.5 : The Structure of Chloroplast

The membranes of chloroplast are rather complex. The lamellae may occur singly, and these are called stromal or intergranal lamellae (intergrana); or they may be stacked like coins, when they are termed granal lamellae (grana), and the individual "coins" are called thylakoids. In cross section, the lamellae look like pairs of membranes separating a narrow internal space (the lumen) from the external stroma.

The membranes of the lamellae contain photosynthetic pigments and an electron transport system. The lumen of the lamellae contains the oxygen generating system. The region around the lamellae (stroma) contains the CO_2 fixing system (dark reactions).

To understand photosynthesis, we need to know a little about photochemistry and the pigments that are involved in converting light into chemical energy. Pigments look coloured because they absorb visible light. We see the colour which they do not absorb: chlorophyll absorbs red and blue light, but not green, so appears green. Light behaves like a stream of particles (quanta) called photons. Pigment molecules absorb light one photon at a time. When a pigment molecule absorbs a photon, it raises its electrons to higher energy levels. The pigment is excited and can perform photochemical reactions. This excitation energy is used in photochemical reactions. The energy (E) in a photon is determined by the wavelength (λ) of the light.

$E = h\,c \times \lambda$

- h, Planck's constant, 6.6×10^{-34} J s.
- c, speed of light, 3×10^{8} m s^{-1}.

Short wavelength photons (blue) have a higher energy than long wavelength (red) photons. Photons promote electrons to excited states: the difference in energy levels must be equal to the energy of the photon. More energetic photons (shorter wavelength) promote electrons to higher energy levels.

The main pigment in green plants is chlorophyll-a. It absorbs red and blue light to make it enter an excited state, and therefore appears green.

Action spectra can be used to show the wavelengths of light that make photosynthesis work. If we compare this to chlorophyll absorption spectrum, we see they are a good match. This implies chlorophyll is the main pigment in photosynthesis (Fig. 13.6).

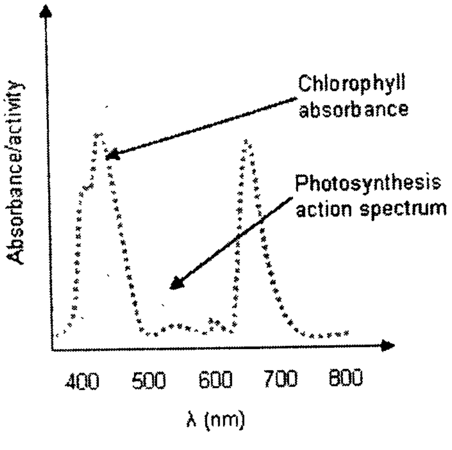


Fig. 13.6 : Absorption spectra of Chlorophyll absorbance and Photosynthesis work

Chlorophyll molecules absorb light as individual photons. Each can cause a single photochemical reaction. If there is no direct photochemical reaction, chlorophyll may lose its excitation energy as heat and red-fluorescence, or by resonance transfer. In fluorescence a high energy (short wavelength) photon is absorbed, which promotes an electron. The electron then drops to the lowest vibrational sub-state of the excited state, releasing heat, before dropping to the ground state, emitting a photon of lower energy (longer wavelength) than that originally absorbed.

Photosynthesis Starts with the Absorption of Light

- Leaves can absorb 90% of the light striking them
- Absorption is by a number of pigments given in Table 13.1:

Table 13.1 : Light Absorption by various photosynthetic Pigments.

Chlorophyll a	▨	Main photosynthetic pigment
Chlorophyll b		Accessory pigment: passes light to chlorophyll a
Xanthophylls		More acessory pigments
Carotene		Another accessory pigment

- Chlorophyll has a light-sensitive ring with a Mg atom in the center. It is anchored in chloroplast membranes by a hydrophobic tail.
- Plants have chlorophylls a & b; algae also have 2 other types (c & d: see table, p. 533 of text).
- Chlorophyll absorbs mostly light in the blue and red regions of the spectrum; it appears green because it does not absorb green light.
    - Light absorption is affected by the environment.
    - Chlorophyll a has an absorption peak either at 680 or 700 nm depending upon what proteins it is associated with (photosystems I & II, see below).

Plants, Cyanobacteria and Algae are Autotrophs

- **Autotrophs** make their own macromolecules and do not require products from other living creatures for life.
- Other types of organisms, including ourselves, are heterotrophs: require products from other species for energy and materials.
- Photosystem I reduces NADP to NADPH. Its reaction centre absorbs maximally at 700 nm, and it is found mostly on intergrana.
- Photosystem II splits water (the Hill reaction). Its reaction centre absorbs maximally at 680 nm, and its is found mostly on grana, associated with light-harvesting complex II. It also contains more chlorophyll-b than PSI.

PSII comes 'earlier' in the reaction series than PSI.

Electrons excited out of the reaction centre in the photosystems are carried along a chain. The chain pumps protons, just like the respiratory complexes, and the electrons are eventually dumped onto NADP to form NADPH. Protons flow back through ATP synthase, generating ATP.

The electron transport chain of photophosphorylation has many similarities to that found in mitochondria: many of the chemical players are the same, and much of the gist will seem familiar but reversed. We will discuss the complexes found in the thylakoid membranes in turn.

Photosystem II

PSII is a water oxidase, and produces 1 PQH_2 (plastoquinol) per water split. A photon of wavelength *c.* 680 nm excites an electron out of the reaction centre of PSII, and the electrons released are transported through the photosystem *via* electron carriers such as phaeophytin and quinone (Q) centres and are dumped onto plastoquinone (PQ to form a semiquinone radical). This generates an extremely high reducing power in a tyrosine residue (called 'Z') of the D1 subunit of PSII, which pulls an electron out of water *via* a manganoprotein to replace that lost from the reaction centre. The water splitting reaction is often termed the Hill reaction.

Plastoquinone and Plastoquinol

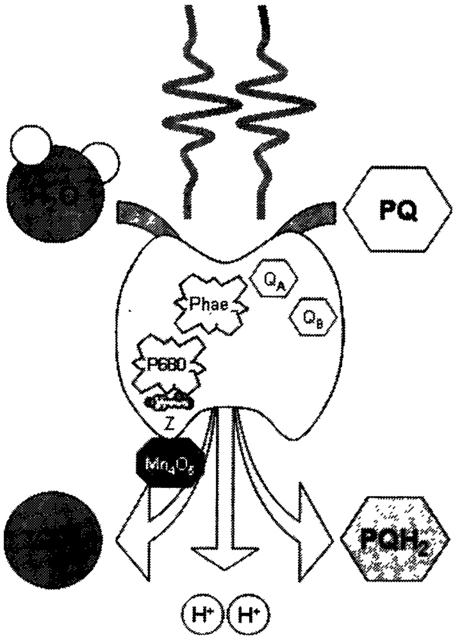


Fig. 13.7 : Function of Plastoquinol in PS II

Ubiquinone (UQ) ferries electrons from PSI to complex III cyt-b_6/f complex. Note the long hydrophobic chain: PQ/PQH_2 can migrate actually dissolved within the membrane (Fig 13.7).

Partial reduction of PQ generates plastosemiquinone radicals ($PQH\cdot$), which are very dangerous and must be rapidly reduced to PQH_2.

PSII feeds into a pool of plastoquinol (UQH_2) actually *inside* the thylakoid membrane.

This is intentionally almost identical to the description of ubiquinol above.

Cyt-b_6/f Complex

Plastocyanin reductase pumps 4 H^+ per PQH_2 from the lumen to the stroma, and makes 2 PC_{RED} per PQH_2 oxidised. The copper in PC goes from Cu^{2+} to Cu^+. (Fig. 13.8)

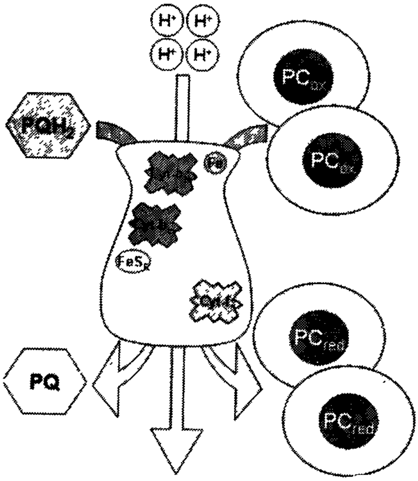


Fig. 13.8 : Function of Cyt-b_6/f complex in PS II

Like cytochrome reductase in mitochondria, this complex runs the Q-cycle, which delivers the two electrons from one PQH_2 to two plastocyanin molecules, which only carry one electron each. For each molecule of PQH_2 that arrives at the complex, one electron is sent back to another PQ, whilst the other is passed on down the chain, to a copper-containing protein called plastocyanin. Two rounds of this pump across 4 protons, and produce two reduced plastocyanin molecules.

Photosystem I

Rarely termed ferredoxin reductase, PSI is excited maximally by 700 nm light, and produces 2 Fd_{RED} per 2 PC_{RED} oxidised. The electrons are passed from P700 through a series of quinones and iron-sulfur centres to the iron-containing protein ferredoxin. Most of the ferredoxin is then immediately passed to a flavin (FMN) cofactor in NADP reductase, generating one of the raw materials for the dark reactions. The electron lost by P700 is replaced by one from plastocyanin. One NADPH is generated per 2 ferredoxin oxidised (Fig. 13.9).

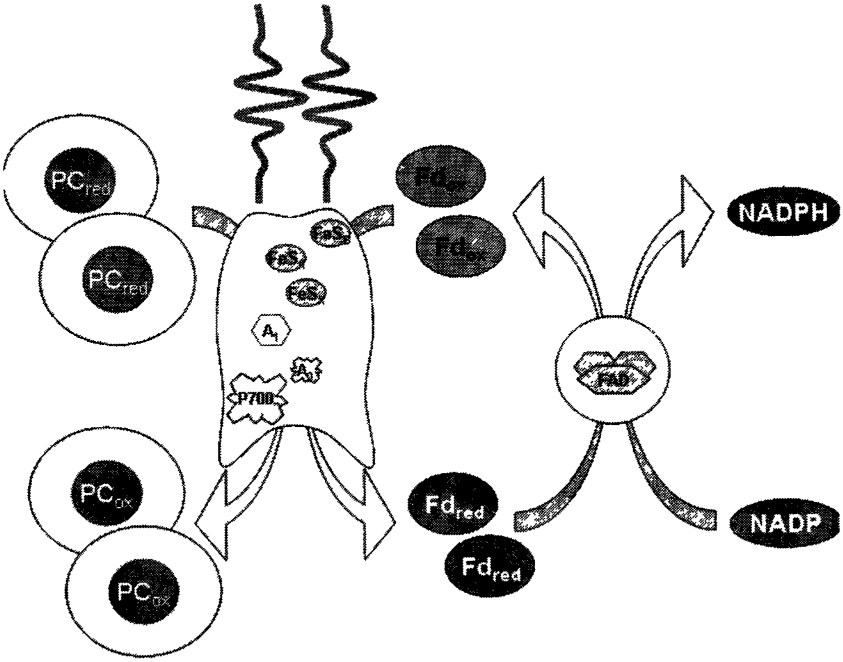


Fig. 13.9 : Functions of PSI to Generate NADPH.

ATP Synthase

Like mitochondria, chloroplasts have an F-type ATPase which generates ATP from a proton gradient. Thylakoids are permeable to Mg^{2+} and Cl^- so this is mostly a concentration effect (pH) rather than an electrical (charge separation) effect. In fact, the pH difference can be very marked: typically the lumen is at pH 4 and the stroma at pH 8.

Many herbicides act by damaging the photosynthetic chain. Atrazine damages the quinone binding area on PSII; paraquat takes electrons from ferredoxin and generates (lethal) hydrogen peroxide. Uncouplers like DNP (obviously) work in chloroplasts as well as mitochondria.

The whole process of the light reactions generates a proton gradient across the membrane. This is used for the chemiosmotic production of ATP. This is called Z-scheme (or straight chain) photo-phosphorylation (Fig. 13.10).

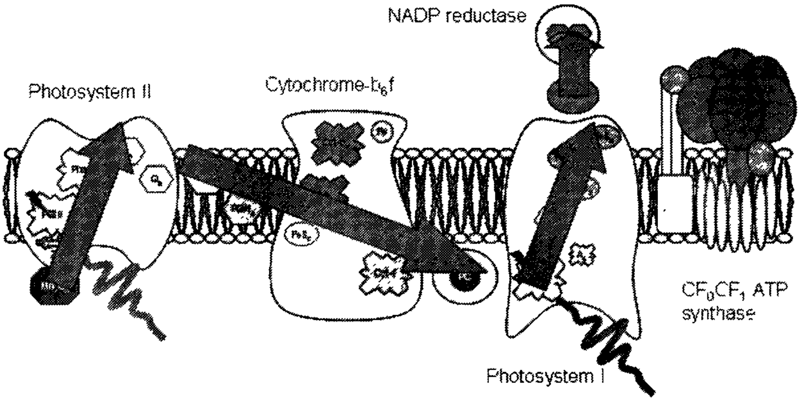

NADP reductase
Photosystem II
Cytochrome-b$_6$f
CF$_0$CF$_1$ ATP synthase
Photosystem I

Fig. 13.10 : ATP Production through Proton Gradient Across the membrane

Energy Budget Per Water Split:

- Water splitting adds 2H$^+$ to the lumen.
- The Q-cycle pumps 4H$^+$ into the lumen.
- NADPH and dark reaction effects cancel.

We need to split two waters per CO_2 fixed, so we double this total to 12 protons, generating 3 or 4 ATP. Only 3 ATP are required per CO_2 in the dark reactions, so we probably make a slight profit of just under 1 ATP per CO_2 over that which is required. However, sometimes, some of the electrons from ferredoxin are fed back to cyt-b$_6$/f, short-circuiting the Z-scheme. This generates ATP *via* the Q-cycle but no NADPH. This is called cyclic phosphorylation and enables the cell to make even more ATP, even when NADPH is not required (Fig. 13.11). Depending on the exact stoichiometry of the various complexes (still not nailed down completely), cyclic phosphorylation may be essential to satisfying the Calvin cycle's requirements.

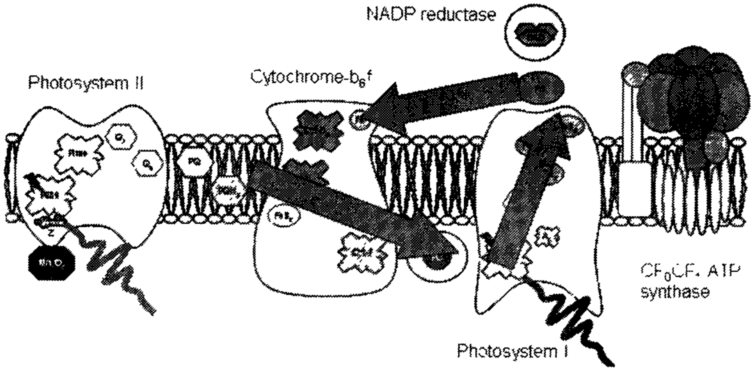

NADP reductase
Photosystem II
Cytochrome-b$_6$f
CF$_0$CF$_1$ ATP synthase
Photosystem I

Fig. 13.11 : Cyclic phosphorylation enables the cell to make more ATP and No NADPH

Dark Reaction of Photosynthesis

The dark reactions are driven by the products of the light reactions.

- The light reactions supply vital materials to the dark reactions:
    - NADPH: supplies activated hydrogens to reduce carbon atoms.
    - ATP: supplies energy to drive several steps.
    - Making a single sugar molecule requires 18 ATPs and 12 NADPHs.

Carbon fixation is catalyzed by Rubisco.

- In the first of the dark reactions CO2 gas is added to a 5 carbon sugar, ribulose bisphosphate.
- This makes a 6 carbon intermediate, which immediately splits into two 3-phosphoglycerates.
- The reaction is catalyzed by ribulose bisphosphate carboxylase, "Rubisco", which is probably the most abundant enzyme on earth (it is 10-25% of leaf protein).

Calvin cycle reactions make sugar from 3-phosphoglycerate.

- The diagram shown in Fig. 13.12 is a simplified outline of the Calvin Cycle reactions which convert CO2 to glucose and other sugars.
- The numbers indicate the numbers of molecules needed to eventually produce 1 glucose molecule.
- The 12 glyceraldehyde-3-phosphates formed are split into 2 groups:
    - 10 are used to regenerate the ribulose bisphosphate and keep the cycle going.
    - 2 are condensed into a glucose molecule.

The rest of the dark reactions have two purposes: firstly to regenerate the acceptor molecule RuBP from PGA; and secondly to generate some sugar profit in the form of glucose, sucrose or starch from PGA.

Two molecules of PGA are formed from the carboxylation of RuBP, one of which is labelled. After a while, other compounds became labelled. Many were known intermediates of glycolysis, and Calvin guessed that the energy from the light reactions of photosynthesis was driving glycolysis backwards to generate glucose from PGA. This is the part that generates our profit. PGA is phosphorylated to 1,3-*bis*-phosphoglycerate by ATP (from light reactions). This is

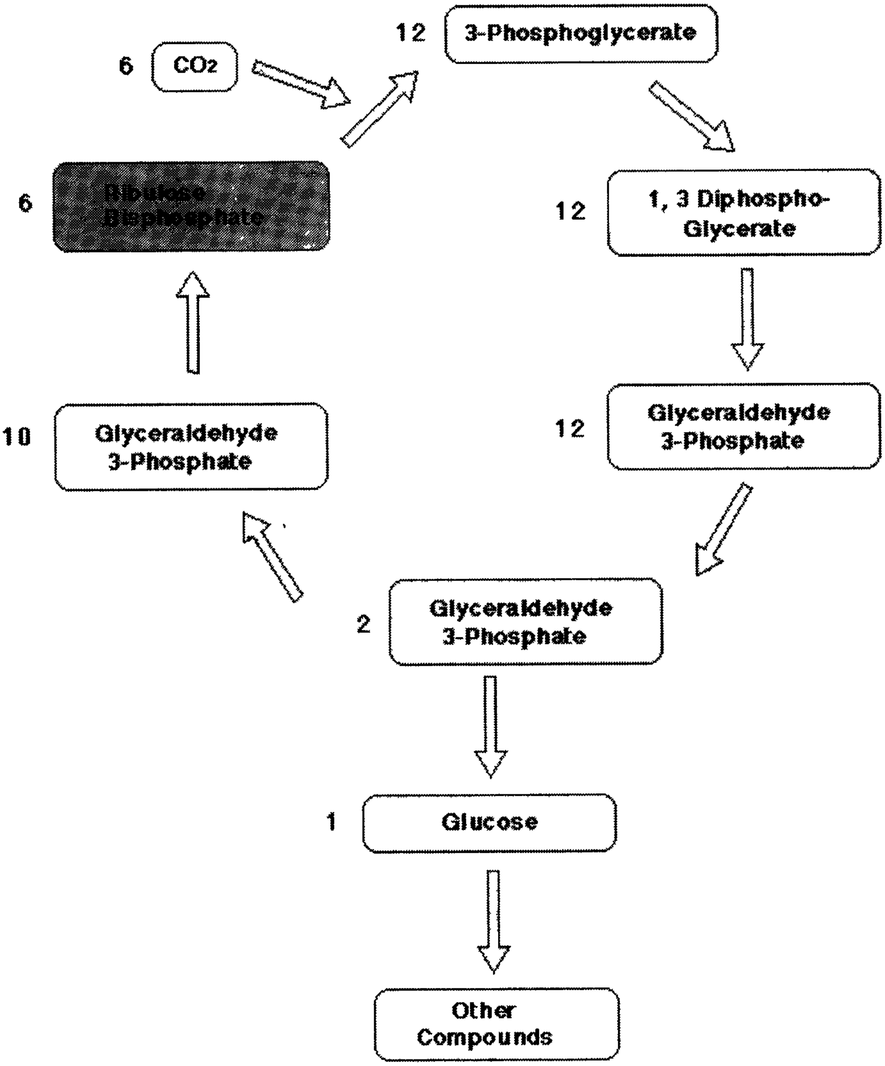

6
CO2
12
3-Phosphoglycerate
6
Ribulose Bisphosphate
12
1, 3 Diphospho-Glycerate
12
Glyceraldehyde 3-Phosphate
10
Glyceraldehyde 3-Phosphate
2
Glyceraldehyde 3-Phosphate
1
Glucose
Other Compounds

Fig. 13.12 : The Calvin Cycle

then reduced to glyceraldehyde-3-phosphate (GAP), using NADPH. GAP dehydrogenase is confined to the chloroplast. Animals also use this pathway to make glucose from Krebs cycle acids by the process termed gluconeogenesis.

$$\text{PGA} + \text{ATP} + \text{NADPH} \longrightarrow \text{GAP} + \text{ADP} + \text{P}_i + \text{NADP}$$

Later, many sugar phosphates were found to be labelled. Everything from trioses to heptuloses: ribose, ribulose, fructose, *etc.* This appeared to be how the dark reactions regenerated RuBP. Sugar (phosphates) found to be synthesized –

- **C3 trioses.**
    - Dihydroxyacetone-P.
- **C4 tetroses**
    - Erythrose-4-P.
- **C5 pentoses**
    - **Ribose-5-P.**
    - Ribulose-5-P.
    - Xylulose-5-P.
- **C6 hexoses**
    - Fructose-1,6-bP
    - Fructose-6-P.
- **C7 heptuloses**
    - Sedoheptulose-1,7-bP
    - Sedoheptulose-7-P

These sugars are all part of the pentose phosphate pathway, which is used to form **ribose** for nucleic acid synthesis in other organisms. The dark reactions have hijacked the pentose phosphate pathway to regenerate RuBP from GAP.

Adding these modified gluconeogenesis and pentose-phosphate pathways together, we get the Calvin cycle. This is an extremely complicated way of regenerating RuBP from GAP, which uses up 1 more ATP per RuBP.

If every 3 RuBP carboxylated, we get 6 PGA. 5 of these are used to regenerate RuBP *via* the Calvin cycle (pentose phosphate pathway). 1 of these is used to generate sugar profit (gluconeogenesis pathway). Hence each turn of the Calvin cycle fixes 6 molecules of CO_2, uses and regenerates 6 RuBP, and generates one hexose profit, using 12 NADPH and F18 ATP from the light reactions (Fig. 13.13).

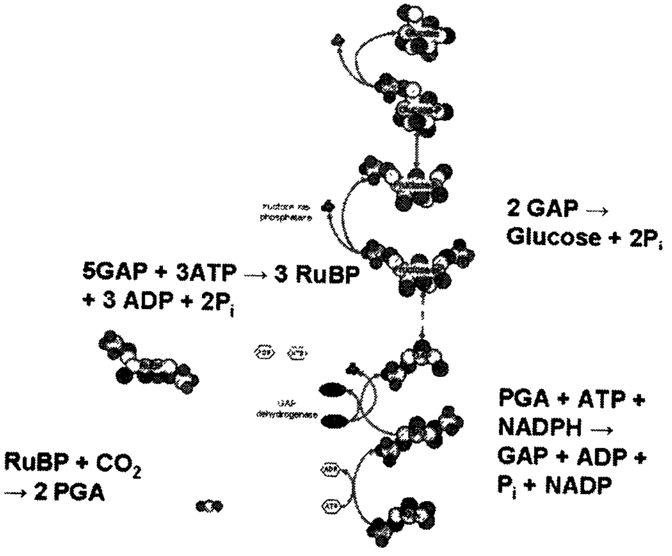

2 GAP → Glucose + $2P_i$
5GAP + 3ATP → 3 RuBP + 3 ADP + $2P_i$
GAP dehydrogenase
PGA + ATP + NADPH → GAP + ADP + P_i + NADP
RuBP + CO_2 → 2 PGA
ADP
ATP

Fig. 13.13 : The dark reactions involve three distinct parts: carbon fixation (Rubisco), gluconeogenesis, and RuBP regeneration (Calvin cycle).

Plants convert glucose to sucrose or starch.

- Plant cells have very little glucose.
- Most of the glucose is immediately converted to:
  - Starch in the chloroplasts and amyloplasts.
  - Sucrose in the cytosol.

Sucrose is transported from the leaf to the rest of the plant through the phloem.

- Vascular plants (flowering plants, gymnosperms, ferns, horsetails, lycopods, Psilotum) have vessels which conduct fluid through the plant (see text p. 555-556 and Ch. 32):
  - Xylem: carries water and minerals from roots upward (transpiration).
  - Phloem: carries sugars and other nutrients from the leaves downward.
- Sucrose is loaded into the phloem by active transport.

Photosynthetic gases enter the leaf through stomata

- In higher plants photosynthesis usually takes place in the leaf.
- Leaves must take up CO2 and give off O2.
- Gases enter leaf through pores called stomata.
- Stomata can open and close (text, p. 701-705).
  - Surrounded by 2 balloon-shaped guard cells.
  - When guard cells swell (by osmosis) the stomata open.
  - When guard cells shrink stomata close.
- Stomata also give up water vapor.
  - Some of this is necessary to pull water through the xylem.
  - Can cause dehydration and wilting if the water is not replaced fast enough.

Plants living in hot environments have evolved photosynthetic variations that minimize water loss.

- Plants in hot climates lose large amounts of water through their stomata when they are open.
- Must be open part of the time to pick up CO_2.
- Two alternative methods of photosynthesis have evolved to conserve water.

- Crassulacean acid metabolism (CAM): CO_2 is taken up at night when it is not as hot (less water loss).
  - The CO_2 taken up is bound to form 4 carbon acids.
  - In the daytime the stomata close and the plant does photosynthesis with the supply of CO_2 taken up during the night.
- C4 Metabolism.

Photorespiration

C4 Metabolism

C4 photosynthesis is an adaptation to growth in hot climates. The first products of photosynthesis are C4 acids: 'normal' plants produce PGA, a 3 carbon acid, and hence are termed C3 plants. C4 photosynthesis has evolved independently in at least 16 families of flowering plants. C4 metabolism is a way of getting round the problem of Rubisco's unfortunate oxygenase activity. C4 plants concentrate CO_2 biochemically in a variety of ways, so Rubisco is exposed to a very high CO_2/O_2 ratio, which inhibits photorespiration.

Some of the most productive crops are C4, including maize and sugarcane; as are some of the worst weeds, such as *Amaranthus* (love-lies-bleeding) and *Tribulus terrestris*. Provided with a high light intensity, their maximum rates of photosynthesis may be double those of C3 plants.

C4 also metabolism reduces *transpiration* by lowering the CO_2 compensation point (Fig. 13.14). Photosynthesis is slower when there is less CO_2 about, and the compensation point is defined as the [CO_2] needed to give net photosynthesis.

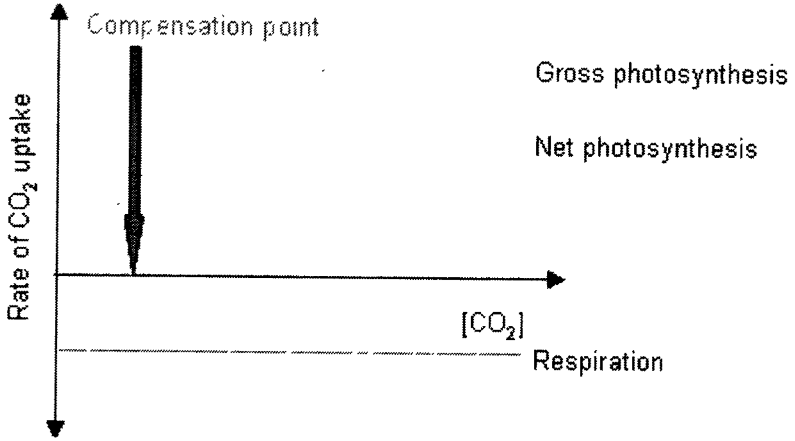


Fig. 13.14 : Compensation Point for C_4 Photosynthesis

At the compensation point, photosynthesis is *just* too slow to outrun (mostly photo) respiration. C3 plants photorespire so much, that their compensation point is *c.* 0.005%. C4 plants do not photorespire, so they have near zero compensation points (Fig 13.14).

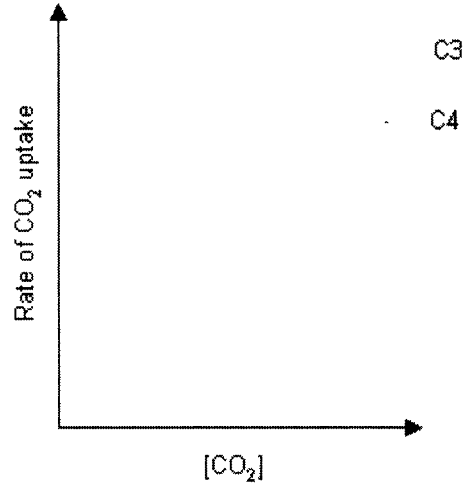


Fig. 13.15 : Comparison of CO_2 uptake during C_3 and C_4 photosynthesis

Stomata close under dry conditions, and when this happens, the levels of CO_2 in the mesophyll air spaces fall towards the compensation point. Since this is lower in C4 plants, they can photosynthesise with narrower stomatal apertures, so only lose half as much water per CO_2 fixed as C3 plants. So although we may regard the C4 syndrome as an adaptation that reduces photorespiration, as far as plants are 'concerned', it is an adaptation to reducing water loss in hot climates.

All C4 plants show Kranz anatomy. The bulk of the photosynthetic tissue is concentrated in two layers around the vascular bundles: the inner bundle-sheath layer and the outer mesophyll layer. C4 leaf veins show up as a darker green than the rest of the leaf: you can usually spot this just by holding a leaf upto the light.

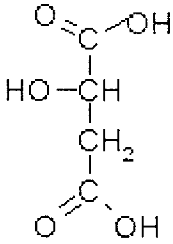
C4 photosynthesis is found in both monocotyledons and dicotyledons. Most of the economically important ones are found in the Poaceae (grasses), and there are some interesting intermediates between C3 and C4, such as *Panicum milioides*. The genus *Atriplex* contains both C3 and C4 types which are still closely enough related to interbreed.

The C4 syndrome was discovery when radioactive $^{14}CO_2$ was fed to sugarcane, and the first products seen were C4 acids, not C3 PGA.

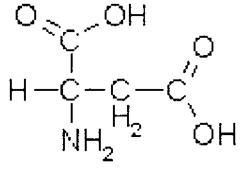
The receptor for CO_2 in C4 plants is not RuBP, but phosphoenolpyruvate (PEP), which is generally carboxylated by PEP carboxylase.

$PEP + CO_2 + H_2O \longrightarrow Oxaloacetate + P_i$

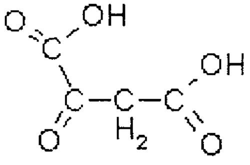
Oxaloacetate is then either reduced to malate; or transaminated to aspartate.



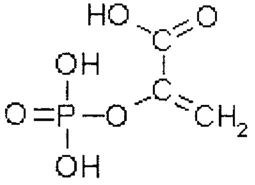
Malate



Aspartate



Oxaloacetate



PEP

CO_2 fixation into C4 acids occurs in the mesophyll. C4 acids are translocated into the bundle-sheath, through plasmodesmata, where they are decarboxylated to give CO_2, and this CO_2 enters the Calvin cycle as normal. The C_3 fragment is returned to the mesophyll and is metabolised back to PEP (Fig. 13.16).

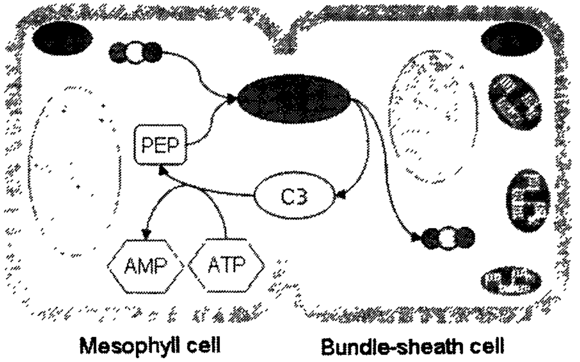
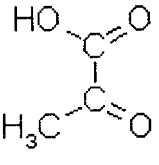


Fig. 13.16 : Photosynthesis in C_4 plants

There are several advantages to C4 metabolism. Firstly CO_2 is released at a much higher concentration (about 10 times atmospheric) in the bundle sheath, where is competes better with oxygen for Rubisco (Rubisco is only found in the bundle sheath). Secondly, it allows net photosynthesis with less carbon dioxide, so the stomatal aperture may be held more nearly closed, and less water is lost. However, there are also disadvantages (or else almost all plants would run C4). The C_3 acids produced by the decarboxylation stage must be converted back to PEP. There are *three* ways of doing this, and they all use up ATP. This means C4 plants need higher light levels to generate the extra ATP. This helps to explain why they are found only in hot, sunny climates.

NADP-ME in maize

This is the first of the three C4 metabolic routes. PEP is carboxylated to oxaloacetate, reduced to malate and shunted into the bundle sheath. The malate is then decarboxylated to pyruvate by NADP-linked malic enzyme in the bundle-sheath chloroplasts. This generates NADPH, so the chloroplasts lack PSII, and make no oxygen *in situ* either, which is an unexpected bonus. Pyruvate is then translocated back to the mesophyll, and phosphorylated back to PEP, using 2 ATP (Fig. 13.17).



Pyruvate

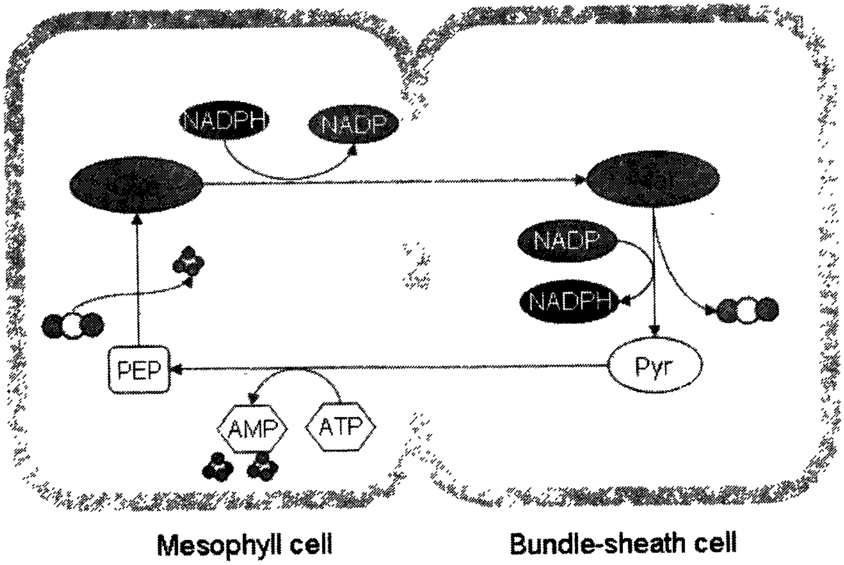


Fig. 13.17 : C4 photosynthesis in Maize

NADP-ME version of C4 metabolism : This uses the equivalent of two ATP per carbon fixation: one ATP is converted to AMP and its two phosphate groups are used, one to phosphorylate pyruvate, the other to phosphorylate phosphate to pyrophosphate, which, rather redundantly is immediately cleaved to two single phosphates. The enzyme performing this double phosphorylation is pyruvate/ orthophosphate dikinase. This is simplified in the diagram above.

NAD-ME in millet

PEP is carboxylated to oxaloacetate, transaminated to aspartate and shunted into the bundle sheath. Aspartate then is transaminated back to oxaloacetate in the mesophyll, reduced to malate by NADH; and decarboxylated to pyruvate by NAD-linked malic enzyme in the bundle-sheath mitochondria. There is no net production of NAD(P)H so the chloroplasts have PSII and may be less effective at inhibiting photorespiration. Pyruvate is phosphorylated in the mesophyll back to PEP, using 2 ATP (Fig. 13.18).

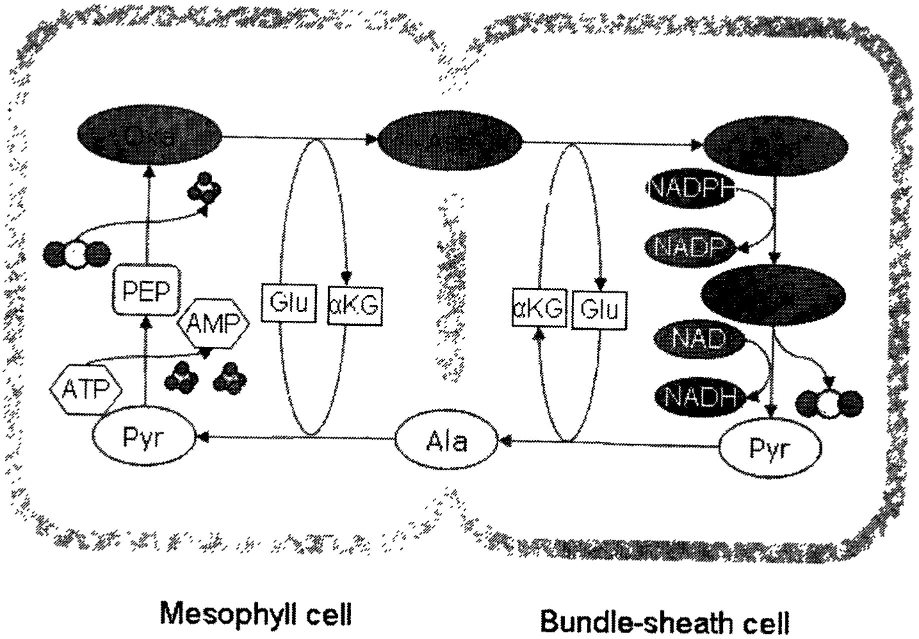


Fig. 13. 18 : Photosynthesis in Millet

NAD-ME version of C4 metabolism : This uses the equivalent of two ATP per carbon fixation for the same reason as was stated in the NADP-ME pathway. Note that there is no net production of NADPH in the bundle sheath, so NAD-ME C4 cannot take advantage of dumping PS-II).

PEP-carboxykinase in Guinea Grass

PEP is carboxylated to oxaloacetate and shunted into the mesophyll. The oxaloacetate is decarboxylated and phosphorylated to PEP in one step using PEP carboxykinase in the cytosol. This only uses 1 ATP and may be more energy-efficient than the other types; however, the reaction is freely reversible, so may not generate such a high level of CO_2 in the bundle-sheath (Fig. 13.19).

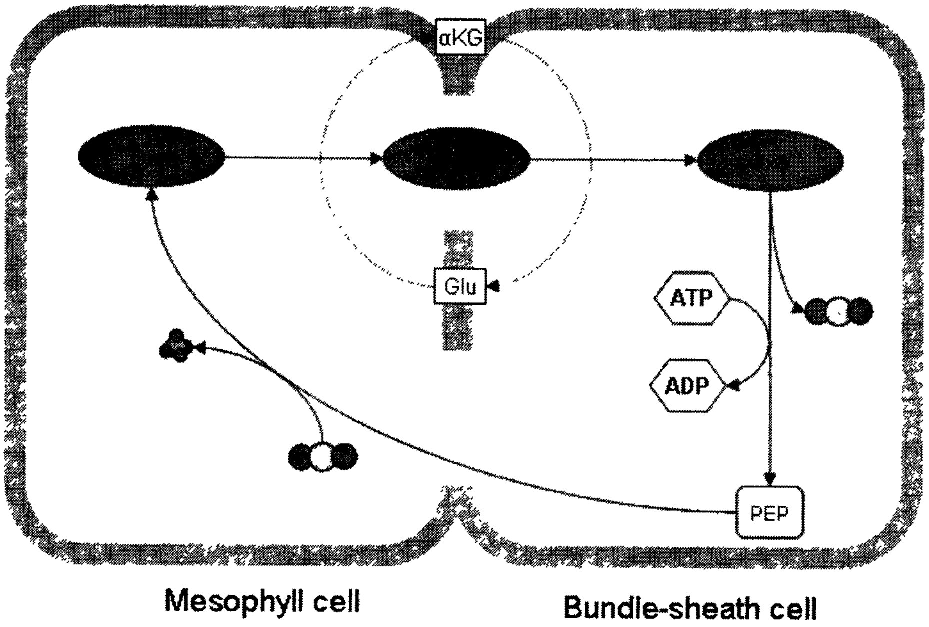


Fig. 13.19 : C4 Photosynthesis in Guinea Grass

PEP-CK version of C4 metabolism. This uses just one ATP per carbon fixation. The little cycle at the top is actually a cheat: two separate transaminase reactions occur: regeneration of glutamate in the mesophyll and ketoglutarate in the bundle sheath requires pyruvate to be transaminated to alanine by glutamate in the bundle sheath, and shunted out to be transaminated back to pyruvate by ketoglutarate in the mesophyll.

CAM Metabolism

There is a second way of inhibiting photorespiration and transpiration, and that is using a process termed CAM. Whereas C4 metabolism physically separates CO_2 fixation and RuBP carboxylation - CO_2 is fixed in the mesophyll, and RuBP is carboxylated in the bundle-sheath - CAM (crassulacean acid metabolism) plants separate these processes *in time*. CAM is even better than C4 at inhibiting photorespiration, and is found in plants from really arid regions.

- Cacti.
- *Crassula*.
- Pineapple.

The biochemistry of CAM is very similar to C4. PEP carboxylase generates oxaloacetate; this is reduced to malate; and malate is split by NADP-malic enzyme to make pyruvate and CO_2. Hence photorespiration is inhibited.

However, the anatomy is nothing special: there is no kranz anatomy; vacuoles in the mesophyll are often large, but not always. So how do they manage to inhibit photorespiration? The clue is in the fact that the pH of the vacuolar sap increases during the day and decreases at night. CAM plants synthesise acids (malate) during the night, and these are used up during the day (Fig. 13.20). This is a temporal separation of fixation and RuBP carboxylation.

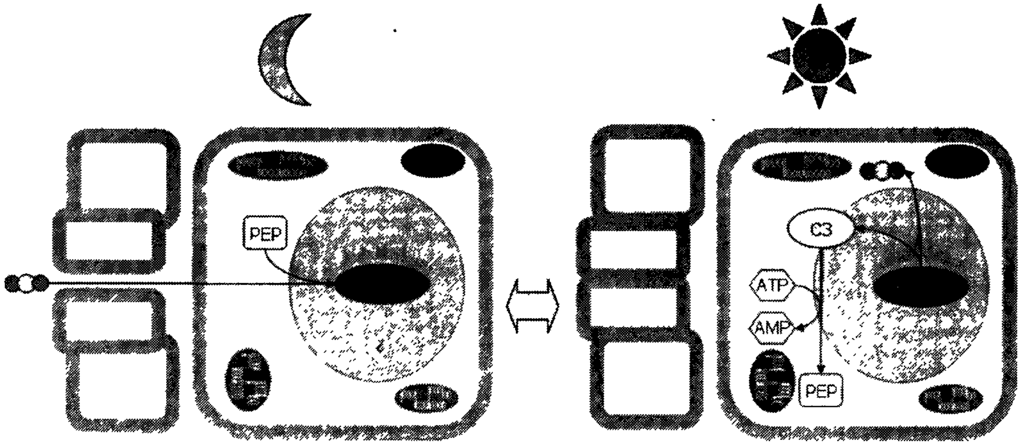


Fig. 13.20 : The CAM Pathway

CO_2 is absorbed by PEP carboxylase during the night when the stomata are open. Malate is stored in the vacuole until morning and used to generate CO_2 during the day. The stomata can stay closed all day, increasing the internal [CO_2], and this reduces photorespiration and water loss hugely.

QUIZ

Identify the letter of the choice that best completes the statement or Answers the question.

1. The process of making food in a plant is called
   - A. Transpiration
   - B. Photosynthesis
   - C. Fertilization
   - D. Respiration

Answer: B

2. Which of these is not needed to make food in a plant?
   - A. Sunlight
   - B. Carbon Dioxide

C. Chlorophyll
D. Flowers

Answer: D

3. The tiny pores or openings in leaves that take in the carbon dixoxide are called
A. Stomata
B. Xylem
C. Phloem
D. Vessels

Answer: A

4. The tubes that bring water from the roots to the leaves are called
A. Stomata
B. Xylem
C. Phloem
D. Vessels

Answer: B

5. The plants give off what that animals need during photosynthesis?
A. Oxygen
B. Carbon dioxide
C. Chlorophyll
D. Water

Answer: A

6. The animals breathe out what that plants need for photosynthesis?
A. Oxygen
B. Carbon dioxide
C. Chlorophyll
D. Water

Answer: B

7. In photosynthesis, excited electrons leave a chlorophyll molecule during:
A. the light-dependent stage
B. cyclic phosphorylation
C. the light-independent stage
D. non-cyclic phosphorylation

Answer: B

8. In the light-independent stage carbohydrate is synthesized from:
A. NAPD
B. ADP
C. ATP
D. PGA

Answer: D

9. Phosphoglyceric acid (PGA) is formed when carbon dioxide is fixed onto the 5C compound:
   A. edenosine triphosphate
   B. ribulose bisphosphate
   C. nicotinamide adenine dinucleotide phosphate
   D. adenosine monophosphate

Answer: B

10. The first stable product of the Calvin Cycle is:
   A. NAPD
   B. PGA
   C. ADP
   D. ATP

Answer: B

11. The greenhouse effect is likely to:
   A. have no effect on the rate of photosynthesis globally
   B. decrease the rate of photosynthesis globally
   C. increase the rate of photosynthesis globally
   D. reduce the rate at which carbon is incorporated into carbohydrate globally

Answer: C

12. If light intensity increases in an atmosphere of excess carbon dioxide, the limiting factor on the rate of photosynthesis is most likely to be:
   A. chlorophyll type
   B. light intensity
   C. carbon dioxide concentration
   D. temperature

Answer: D

13. The two principal stages of photosynthesis are:
   A. cyclic and non-cyclic phosphorylation
   B. the light-dependent and light-independent reactions
   C. photolysis and ATP synthesis
   D. photosystem I and photosystem II

Answer: B

14. In photosynthesis, carbohydrates are produced during:
   A. the light-dependent stage
   B. the light-independent stage
   C. non-cyclic phosphorylation
   D. cyclic phosphorylation

Answer: B

15. In photosynthesis, oxygen is produced during:
   A. the light-dependent stage

B. the light reactions
C. non-cyclic phosphorylation
D. cyclic phosphorylation

Answer: C

16. In photosynthesis, carbon dioxide is fixed during:
A. the light-dependent stage
B. the light-independent stage
C. cyclic phosphorylation
D. non-cyclic phosphorylation

Answer: B

17. Ribulose bisphosphate inhibits rubisco activity by:
A. covalent modification of the enzyme to its inactive form
B. product inhibition
C. incorporating carbon dioxide to form 2 molecules of 3-phosphoglycerate
D. binding more tightly to the inactive form of rubisco

Answer: D

18. Chlorophyll contains all of the following except:
A. tetrapyrroles substituents
B. phytol
C. magnesium
D. iron

Answer: D

19. 2400 chlorophyll molecules produce 1 oxygen molecule. This implies:
A. All chlorophyll molecules harvest light and through a photochemical reaction convert light energy into chemical energy.
B. Some chlorophyll molecules are inactive but most energy is emitted as fluorescence.
C. Most chlorophyll molecules harvest light and transfer the energy to reaction centers that perform a photochemical event.
D. Most chlorophyll molecules are incorporated in a reaction center, but only a few chlorophyll molecules are capable of harvesting light.

Answer: C

20. The direct electron acceptor from P680 * is:
A. the Mn-complex
B. pheophytin
C. plastoquinone
D. water

Answer: B

21. The ATP synthase is located in:
A. the chloroplast membrane

B. the thylakoid membrane
C. the intermembrane space
D. the stroma

Answer: B

22. In the electron flow from H_2O to $NADP^+$, the electron is transferred to each of the following components in what order?
A. Photosystem II, plastoquinone, cyt b/fm plastocyanin, Photosystem I
B. Photosystem I, plastocyanin, cyt b/f, plastoquinone, Photosystem II
C. Photosystem I, plastoquinone, plastocyanin, cyt b/f, Photosystem II
D. Photosystem II, plasotcyanin, cyt b/f, plastoquinone, Photosystem I

Answer: A

23. Photosystem I activation directly produces:
A. water
B. oxygen
C. NADPH
D. NADP+

Answer: C

24. All of the following events are responsible for creating a proton gradient except:
A. hydrolysis by Photosystem II
B. oxidation-reduction of plastoquinone pool
C. NADP+ reduction by Photosystem I
D. ATP production by ATP synthase

Answer: C

25. Cyclic photophosphorylation produces:
A. ATP, O2, NADPH
B. ATP, O2
C. ATP, NADPH
D. ATP

Answer: D

26. The primary product of carbon dioxide fixation is:
A. ribulose-1,5-bisphosphate
B. 2-carboxy, 3-keto-arabinitol
C. 3-phosphoglycerate
D. glyceraldehyde-3-phosphate

Answer: C

27. The C-4 carbon dioxide carrier is:
A. oxaloacetate
B. glyoxylate
C. apartate
D. phosphoenolpyruvate

Answer: C

28. Oxidation of chlorophyll results in:
   - A. Mg +
   - B. Chl.* +
   - C. Mn +
   - D. Fe 2+

Answer: B

29. During the light-dependent reactions of photosynthesis, __________ passes electrons to an Electron Transport Chain.
   - A. chlorophyll a
   - B. chlorophyll b
   - C. carotenoid
   - D. water

Answer: A

30. A chlorophyll "a" molecule consists of all the following structural components except:
   - A. alternating single and double bonds
   - B. phytol tail
   - C. stroma
   - D. central magnesium atom

Answer: C

31. One important contribution of water to the light-dependent reactions is that it:
   - A. provides hydrogen ions to PI
   - B. provides carbon dioxide to PI
   - C. provides photons to PII
   - D. provides electrons to PII

Answer: D

32. The major product(s) of the light-independent reactions is/are:
   - A. NADPH, ATP
   - B. NADP+, ATP, oxygen
   - C. water, H+
   - D. sugar

Answer: D

33. C_4 plants utilize the enzyme ______________ to initially fix carbon dioxide in the__________ cells.
   - A. PEP carboxylase; mesophyll
   - B. RuBP carboxylase; bundle sheath
   - C. ATP synthase; stroma
   - D. PEP carboxylase; matrix

Answer: A

34. During cyclic photophosphorylation:
    A. no oxygen is produced
    B. no ATP is produced
    C. no NADPH is produced
    D. A and C

Answer: D

35. The light-dependent reactions occur in the ______; whereas the light-independent reactions occur in the _______.
    A. stroma; thylakoid
    B. stroma; matrix
    C. thylakoid; stroma
    D. matrix; cytoplasm

Answer: C

36. The first stable 3-carbon organic molecule produced by the C_3 (Calvin) cycle is:
    A. PGA
    B. PGAL
    C. PEP
    D. glucose

Answer: A

37. How many carbon dioxide molecules must be "fixed" in the C3 cycle to produce 1 molecule of glucose?
    A. 2
    B. 4
    C. 6
    D. 8

Answer: C

38. Photosynthesis occurs most efficiently when plants are exposed to ______ light.
    A. green
    B. orange
    C. yellow
    D. blue

Answer: D

39. The major pigments responsible for photosynthesis in green plants are:
    A. chlorophylls
    B. carotenoids
    C. anthocyanins
    D. hemoglobins

Answer: A

40. The electron transport chain in the thylakoid membrane passes electrons from carrier to carrier. In the process, energy is released and is used to pump ________ from the stroma to the thylakoid interior.
   A. glucose
   B. oxygen
   C. electrons
   D. protons

Answer: D

41. Electrons that pass down the ETS of both Photosystem I and II are finally picked up by:
   A. glucose
   B. NAD+
   C. NADH
   D. NADP+

Answer: D

42. Stomata open to allow carbon dioxide into the leaf. The stomata open when surrounding guard cells fill with _____ and bend allowing a space (the stoma) to form between them.
   A. water
   B. air
   C. chlorophyll
   D. electrons

Answer: B

43. Glucose produced by photosynthesis can be saved by the plant as _____ (for food storage) and _______ (for structural material).
   A. protein; starch
   B. cellulose; sucrose
   C. starch; cellulose
   D. glycogen; lipid

Answer: B

44. The process by which energy is obtained directly from sunlight and stored in organic compounds is called...
   A. crassulacean acid metabolism
   B. photosynthesis
   C. transpiration
   D. respiration

Answer: B

45. Organisms that manufacture their own food from inorganic substances and energy (photosynthesize and/or chemosythesize) are calleD...
   A. tertiary consumers
   B. fungi

C. protists
D. heterotrophs

Answer: D

46. All living things *indirectly* get their food from...
A. photosynthesis
B. only chemosynthesis
C. particle spin
D. pigments

Answer: A

47. What kinds of organisms perform cellular respiration?
A. crustaceans only
B. plants only
C. animals only
D. none of them

Answer: B

48. Which part of a typical land plant is most directly involved with the process of transpiration?
A. chloroplasts
B. phloem
C. cambium
D. stomata

Answer: D

49. Which type of organism synthesizes organic materials from inorganic raw materials?
A. autotroph
B. heterotroph
C. parasite
D. saprophyte

Answer: A

50. Which energy conversion occurs during the process of photosynthesis?
A. chemical bond energy to light energy
B. light energy to mechanical energy
C. mechanical energy to radiant energy
D. light energy to chemical bond energy

Answer: D

51. The raw materials for photosynthesis are
A. oxygen and carbon dioxide
B. carbon dioxide and water
C. oxygen and water
D. sugar and carbon dioxide

Answer: B

52. The size of the stomata in a leaf are controlled by the
    A. xylem cells
    B. guard cells
    C. phloem cells
    D. cambium cells

Answer: B

53. The conversion of light energy into chemical bond energy can be best carried on by cells which contain
    A. centrioles
    B. glycogen
    C. contractile vacuoles
    D. chlorophyll

Answer: D

54. Which process is most closely associated with the activity of the guard cells?
    A. digestion
    B. reproduction
    C. locomotion
    D. transpiration

Answer: D

55. Plants which grow in dry environments would most likely have
    A. small leaves with few stomates
    B. stomata which are permanently open
    C. large leaves with many stomata
    D. stomata in their stems and roots

Answer: A

56. During photosynthesis in a bean plant, which wavelength of light is least effective?
    A. red
    B. blue
    C. green
    D. violet

Answer: C

57. The chief purpose of the photosynthetic process is considered to be the
    A. production of water
    B. production of carbon dioxide
    C. production of chlorophyll
    D. the conversion of light energy to chemical energy

Answer: D

58. A plant that lacks chloroplasts does NOT
    A. give off oxygen

B. give off carbon dioxide
C. take in water
D. take in food

Answer: A

59. Which substances enter a green plant chiefly through its leaves?
A. oxygen and minerals
B. . water and minerals
C. water and carbon dioxide
D. carbon dioxide and oxygen

Answer: D

60. Which of the following is C_4 plants ?
A. Wheat
B. Maize
C. Groundnut
D. Pigeonpea

Answer: B

Complete each sentence or statement.

1. The ________ is the sequence of photosynthetic reactions in which ribulose-5-phosphate is carboxylated, converted to three-carbon carbohydrate precursors, and regenerated.

Answer: Calvin cycle

2. Fluorescence is a mode of decay of an excited molecule, in which electronic energy is emitted in the form of a/an ________.

Answer: photon

3. The ________ is the gel-like solution of enzymes and small molecules in the interior of the chloroplast.

Answer: stroma

4. ________ are the photosynthetic reactions in which light energy is absorbed and used to generate NADPH and ATP.

Answer: Light reactions

5. The membranous structure in the interior of a chloroplast that is the site of the light reactions of photosynthesis is the ________.

Answer: thylakoid

6. The ________ is the ratio of carbon atoms fixed or oxygen molecules produced to the number of photons absorbed by the photosynthetic machinery.

Answer: quantum yield

7. ________ is a mode of decay of an energetically excited molecule, in which electronic energy is transferred to a nearby unexcited molecule.

Answer: Exciton transfer

8. The incorporation of carbon dioxide into biologically useful organic molecules is called ________.

Answer: carbon fixation

9. The photosynthetic reactions in which NADPH and ATP produced by the light reactions are used to incorporate carbon dioxide into carbohydrates are called ________.

Answer: dark reactions

10. The light-driven incorporation of carbon dioxide into organic compounds is called ________.

Answer: photosynthesis

11. A/An ________ is a molecule that transfers its absorbed energy to other pigment molecules and eventually to a photosynthetic reaction center.

Answer: antenna pigment

12. ________ is a mode of decay of an excited molecule that occurs through the transfer of an electron to another molecule.

Answer: Photooxidation

13. A daily oscillation in a biochemical process that is coordinated with the daily light-dark cycle is called a ________.

Answer: Circadian cycle

14. A/An ________ is the plant organelle where photosynthesis takes place.

Answer: chloroplast

15. A light-absorbing molecule, or pigment, is a/an ________.

Answer: photoreceptor

16. The ________ is a pigment-containing protein that collects light energy in order to transfer it to a photosynthetic reaction center.

Answer: light-harvesting complex

17. A/An ________ is a diagram indicating the electron carriers and their reduction potentials in the photosynthetic electron-transport system of plants and cyanobacteria.

Answer: Z-scheme

18. The chlorophyll group(s) where photooxidation takes place is/are called a/an ________.

Answer: reaction center

“This Page is Intentionally Left Blank”

General Biochemistry Glossary

A form. A duplex DNA structure with right-handed twisting in which the planes of the base pairs are tilted about 70° with respect to the helix axis.

Acetal. The product formed by the successive condensation of two alcohols with a single aldehyde. It contains two ether-linked oxygens attached to a central carbon atom.

Acetyl CoA. Acetyl-coenzyme A, a high-energy ester of acetic acid that is important both in the tricarboxylic acid cycle and in fatty acid biosynthesis.

Actin. A protein found in combination with myosin in muscle and also found as filaments constituting an important part of the cytoskeleton in many eukaryotic cells.

Actinomycin D. An antibiotic that binds to DNA and inhibits RNA chain elongation.

Activated complex. The highest free energy state of a complex in going from reactants to products.

Active site. The region of an enzyme molecule that contains the substrate binding site and the catalytic site for converting the substrate(s) into product(s).

Active transport. The energy-dependent transport of a substance across a membrane.

Adenine. A purine base found in DNA or RNA.

Adenosine diphosphate (ADP). The nucleotide formed by adding a pyrophosphate group to the 5'-OH group of adenosine.

Adenosine triphosphate (ATP). The nucleotide formed by adding yet another phosphate group to the pyrophosphate group on ADP.

Adenosine. A purine nucleoside found in DNA, RNA, and many cofactors.

Adenylate cyclase. The enzyme that catalyzes the formation of cyclic 3',5' adenosine monophosphate (cAMP) from ATP.

Adipocyte. A specialized cell that functions as a storage depot for lipid.

Aerobe. An organism that utilizes oxygen for growth.

Affinity chromatography. A column chromatographic technique that employs attached functional groups that have a specific affinity for sites on particular proteins.

Alcohol. A molecule with a hydroxyl group attached to a carbon atom.

Aldehyde. A molecule containing a doubly bonded oxygen and a hydrogen attached to the same carbon atom.

Alginate. Component of the cell walls of many rhodophytes and kelps. Alginates have an affinity for water, and so help to slow dessication when the algae are exposed to the air; they are commercially important in the production of paper, toothpaste, beer, and frozen foods.

Alleles. Alternative forms of a gene.

Allosteric enzyme. An enzyme whose active site can be altered by the binding of a small molecule at a nonoverlapping site.

Amino Acid. Unit molecule from which proteins are constructed by polymerization.

Angstrom (Å). A unit of length equal to 10^{-10} m.

Anomers. The sugar isomers that differ in configuration about the carbonyl carbon atom. This carbon atom is called the anomeric carbon atom of the sugar.

Antibiotic. A natural product that inhibits bacterial growth (is bacteriostatic) and sometimes results in bacterial death (is bacteriocidal).

Antibody. A specific protein that interacts with a foreign substance (antigen) in a specific way.

Anticodon. A sequence of three bases on the transfer RNA that pair with the bases in the corresponding codon on th messenger RNA.

Antigen. A foreign substance that triggers antibody formation and is bound by the corresponding antibody.

Antiparallel α-pleated sheet (β-sheet). A hydrogen bonded secondary structure formed between two or more extended polypeptide chains.

Apoactivator. A regulatory protein that stimulates transcription from one or more genes in the presence of a coactivator molecule.

Asexual reproduction. Growth and cell duplication that does not involve the union of nuclei from cells of opposite mating types.

Asymmetric carbon. A carbon that is covalently bonded to four different groups.

ATP "Adenosine triphosphate". A relatively stable, high energy molecule used to fuel chemical reactions within cells.

Attenuator. A provisional transcription stop signal.

Autoradiography. The technique of exposing film in the presence of disintegrating radioactive particles. Used to obtain information on the distribution of radioactivity in a gel or a thin cell section.

Autoregulation. The process in which a gene regulates its own expression.

Autotroph. An organism that can form its organic constituents from CO_2.

Auxin. A plant growth hormone usually concentrated in the apical bud.

Auxotroph. A mutant that cannot grow on the minimal medium on which a wild-type member of the same species can grow.

Avogadro's number. The number of molecules in a gram molecular weight of any compound (6.023×10^{23}).

B cell. One of the major types of cells in the immune system. B cells can differentiate to form memory cells or antibody-forming cells.

B form. The most common form of duplex DNA, containing a right-handed helix and about 10 (10.5 exactly) base pairs per turn of the helix axis.

Base analog. A compound, usually a purine or a pyrimidine, that differs somewhat from a normal nucleic acid base.

Base stacking. The close packing of the planes of base pairs, commonly found in DNA and RNA structures.

Base. The adenine, guanine, cytosine or thymine group attached to a nucleotide or nucleoside. Also may be used to refer to a nucleic acid unit within a polynucleotide chain, as when a gene is said to be 2000 bases long.

Beta-bend (β-bend) or turn. A characteristic way of turning an extended polypeptide chain in a different direction, involving the minimum number of residues, and held together by hydrogen bonding.

Beta-oxidation (β-oxidation). Oxidative degradation of fatty acids that occurs by the successive oxidation of the □-carbon atom.

Beta-sheet (β-sheet). A sheetlike structure formed by the interaction between two or more extended polypeptide chains.

Bidirectional replication. Replication in both directions away from the origin, as opposed to replication in one direction only (unidirectional replication).

Bilayer. A double layer of lipid molecules with the hydrophilic ends oriented outward, in contact with water, and the hydrophobic parts oriented inward.

Bile salts. Derivatives of cholesterol with detergent properties that aid in the solubilization of lipid molecules in the digestive tract.

Biochemical pathway. A series of enzyme-catalyzed reactions that results in the conversion of a precursor molecule into a product molecule.

Biochemistry. The study of those molecules used and manufactured by living things.

Bioluminescence. The production of light by a chemical reaction within an organism. The process occurs in many bacteria and protists, as well as certain animals and fungi.or The production of light by a biochemical system.

Blastoderm. The stage in embryogenesis when a unicellular layer at the surface surrounds the yolk mass.

Bond energy. The energy required to break a bond.

Branchpoint. An intermediate in a biochemical pathway that can follow more than one route in following steps.

Brevitoxin. Neurotoxin produced by the dinoflagellate Ptychodiscus brevis.

Buffer. A conjugate acid-base pair that is capable of resisting changes in pH when acid or base is added to the system. This tendency will be maximal when the conjugate forms are present in equal amounts.

Calcite. A common crystalline form of natural calcium carbonate, caco3, that is the basic constituent of limestone, marble, and chalk. Also called calcspar.

Calcium Carbonate. A "salt" used by many marine invertebrates, such as corals and echinoderms, and by protists, such as coccolithophorids, to construct their exoskeletons.

cAMP. 3',5' cyclic adenosine monophosphate. The cAMP molecule plays a key role in metabolic regulation.

CAP. The catabolite gene activator protein, sometimes incorrectly referred to as the CRP protein. The latter term, in small letters (crp), should be used to refer to the gene but not to the protein.

Capping. Covalent modification involving the addition of a modified guanidine group in a 5'-5" linkage. It occurs only in eukaryotes, primarily on mRNA molecules.

Carbohydrate. A polyhydroxy aldehyde or ketone. or Class of biochemical compounds which includes sugars, starch, chitin, and steroids.

Carboxylic acid. A molecule containing a carbon atom attached to a hydroxyl group and to an oxygen atom by a double bond.

Carcinogen. A chemical that can cause cancer.

Carotenoids. Lipid-soluble pigments that are made from isoprene units.

Catabolism. That part of metabolism that is concerned with degradation reactions.

Catabolite repression. The general repression of transcription of genes associated with catabolism that is seen in the presence of glucose.

Catalyst. A compound that lowers the activation energy of a reaction without itself being consumed.

Catalytic site. The site of an enzyme involved in the catalytic process.

Catenane. An interlocked pair of circular structures, such as covalently closed DNA molecules.

Catenation. The linking of molecules without any direct covalent bonding between them, as when two circular DNA molecules interlock like the links in a chain.

cDNA. Complementary DNA, made in vitro from the mRNA by the enzyme reverse transcriptase using deoxyribonucleotide triphosphates. Unlike mRNA, cDNA can be easily propagated and sequenced.

Cell commitment. That stage in a cell's life when it be comes committed to a certain line of development.

Cell cycle. All of those stages that a cell passes through from one cell generation to the next.

Cell line. An established clone originally derived from a whole organism through a long process of cultivation.

Cell lineage. The pedigree of cells resulting from binary fission.

Cell wall. A tough outer coating found in many plant, fungal, and bacterial cells that accounts for their ability to withstand mechanical stress or abrupt changes in osmotic pressure. Cell walls always contain a carbohydrate component and frequently also a peptide and a lipid component.

Cellulose. Carbohydrate polymer of the simple sugar glucose. It is found in the cell walls of plants and green algae, as well as dinoflagellates. Cellulose is the most abundant compound on earth that is manufactured by living things.

Chelate. A molecule that contains more than one binding site and frequently binds to another molecule through more than one binding site at the same time.

Chemiosmotic coupling. The coupling of ATP synthesis to an electrochemical potential gradient across a membrane.

Chimeric DNA. Recombinant DNA whose components originate from two or more different sources.

Chiral compound. A compound that can exist in two forms that are non-superimposable images of one another.

Chitin. N. A carbohydrate polymer found in the cell walls of fungi and in the exoskeletons of arthropods, which provides strength for support and protection; chitinous- adj.

Chlorophyll. A green photosynthetic pigment that is made of a magnesium dihydroporphyrin complex. or The green-colored pigment that absorbs light during photosynthesis, often found in plants, algae, and some bacteria; it includes a porphyrin ring, and often has a long hydrophobic tail.

Chloroplast. A chlorophyll-containing photosynthetic organelle, found in eukaryotic cells, that can harness light energy.

Chromatin. The nucleoprotein fibers of eukaryotic chromosomes.

Chromatography. A procedure for separating chemically similar molecules. Segregation is usually carried out on paper or in glass or metal columns with the help of different solvents. The paper or glass columns contain porous solids with functional groups that have limited affinities for the molecules being separated.

Chromosome puff. A swollen region of a giant chromosome; the swelling reflects a high degree of transcription activity.

Chromosome. A thread-like structure, visible in the cell nucleus during metaphase, that carries the hereditary information.

Cis dominance. Property of a sequence or a gene that exerts a dominant effect on a gene to which it is linked.

Cistron. A genetic unit that encodes a single polypeptide chain.

Citric acid cycle. See tricarboxylic acid (TCA) cycle.

Clone. One of a group of genetically identical cells or organisms derived from a common ancestor.

Cloning vector. A self-replicating entity to which foreign DNA can be covalently attached for purposes of amplification in host cells.

Coactivator. A molecule that functions together with a protein apoactivator. For example, cAMP is a coactivator of the CAP protein.

Codon. In a messenger RNA molecule, a sequence of three bases that represents a particular amino acid.

Coenzyme. An organic molecule that associates with enzymes and affects their activity.

Cofactor. A small molecule required for enzyme activity. It could be organic in nature, like a coenzyme, or inorganic in nature, like a metallic cation.

Collagen. Long proteins whose structure is wound into a triple helix. The resulting fibers have a high tensile strength. Collagen is a primary component of mammalian hair.

Complementary base sequence. For a given sequence of nucleic acids, the nucleic acids that are related to them by the rules of base pairing.

Configuration. The spatial arrangement in which atoms are covalently linked in a molecule.

Conformation. The three-dimensional arrangement adopted by a molecule, usually a complex macromolecule. Molecules with the same configuration can have more than one conformation.

Consensus sequence. In nucleic acids, the "average" sequence that signals a certain type of action by a specific protein. The sequences actually observed usually vary around this average.

Constitutive enzymes. Enzymes synthesized in fixed amounts, regardless of growth conditions.

Cooperative binding. A situation in which the binding of one ligand to a macromolecule favors the binding of another. For example, DNA cooperatively binds histone molecules, and hemoglobin cooperatively binds oxygen molecules.

Coordinate induction. The simultaneous expression of two or more genes.

Cosmid. A DNA molecule with cos ends from lambda-bacteriophage that can be packaged in vitro into a virus for infection purposes .

Cot curve. A curve that indicates the rate of DNA-DNA annealing as a function of DNA concentration and time.

Cytidine. A pyrimidine nucleoside found in DNA and RNA.

Cytochromes. Heme-containing proteins that function as electron carriers in oxidative phosphorylation and photosynthesis.

Cytokinin. A plant hormone produced in root tissue.

Cytoplasm. The contents enclosed by the plasma (or cytoplasmic) membrane, excluding the nucleus.

Cytosine. A pyrimidine base found in DNA and RNA.

Cytoskeleton. The filamentous skeleton, formed in the eukaryotic cytoplasm, that is largely responsible for controlling cell shape.

Cytosol. The liquid portion of the cytoplasm, including the macromolecules but not including the larger structures like subcellular organelles or cytoskeleton.

D loop. An extended loop of single-stranded DNA displaced from a duplex structure by an oligonucleotide.

Dalton. A unit of mass equivalent to the mass of a hydrogen atom (1.66 x 10^{-24} g)

Dark reactions. Reactions that can occur in the dark, in a process that is usually associated with light, such as the dark reactions of photosynthesis.

De novo pathway. A biochemical pathway that starts from elementary substrates and ends in the synthesis of a biochemical.

Deamination. The enzymatic removal of an amine group, as in the deamination of an amino acid to an alpha keto acid.

Dehydrogenase. An enzyme that catalyzes the removal of a pair of electrons (and usually one or two protons) from a substrate molecule.

Denaturation. The disruption of the native folded structure of a nucleic acid or protein molecule; may be due to heat, chemical treatment, or change in pH.

Density-gradient centrifugation. The separation, by centrifugation, of molecules according to their density, in a gradient varying in solute concentration.

Dialysis. Removal of small molecules from a macromolecule preparation by allowing them to pass across a semipermeable membrane.

Diauxic growth. Biphasic growth on a mixture of two carbon sources in which one carbon source is used up before the other one. For example, in the presence of glucose and lactose, *E. coli* will utilize the glucose before the lactose.

Difference spectra. Plots comparing the absorption spectra of a molecule or an assembly of molecules in different states, for example, those of mitochondria under oxidizing or reducing conditions.

Differential centrifugation. Separation of molecules and/or organelles by sedimentation rate.

Differentiation. A change in the form and pattern of a cell and the genes it expresses as a result of growth and replication, usually during development of a multicellular organism. Also occurs in microorganisms (e.g. in sporulation).

Dimer. Structure resulting from the association of two subunits.

Dinosteranes/Dinosteroids. Chemicals found in dinoflagellates, which have been useful in documenting their existence early in the fossil record.

Diploid cell. A cell that contains two chromosomes (2N) of each type.

Dipole. A separation of charge within a single molecule.

Directed mutagenesis. In a DNA sequence, an intentional alteration that can be genetically inherited.

Dissociation constant. An equilibrium constant for the dissociation of a molecule into two parts (e.g., dissociation of acetic acid into acetate anion and a proton); K_d.

Disulfide bridge. A covalent linkage formed by oxidation between two cysteine SH groups either in the same polypeptide chain or in different polypeptide chains. Reversible by adding reducing agents.

DNA cloning. The propagation of individual segments of DNA as clones.

DNA library. A mixture of clones, each containing a cloning vector and a segment of DNA from a source of interest.

DNA polymerase. An enzyme that catalyzes the formation of 3'-5' phosphodiester bonds from deoxyribonucleotide triphosphates.

DNA. Deoxyribonucleic acid. A polydeoxyribonucleotide in which the sugar is deoxyribose; the main repository of genetic information in all cells and most viruses. or "Deoxyribonucleic acid". The nucleic acid which carries the genetic code of an organism. It is the primary component of chromosomes.

Domain. A segment of a folded protein structure showing conformational integrity. A domain could include the entire protein or just a fraction of the protein. Some proteins, such as antibodies, contain many structural domains.

Dominant. Describing an allele whose phenotype is expressed regardless of whether the organism is homozygous or heterozygous for that allele.

Double helix. A structure in which two helically-twisted polynucleotide strands are held together by hydrogen bonding and base stacking.

Duplex. Same as double helix.

Dyad symmetry. Property of a structure that can be rotated by 180° to produce the same structure.

Ecdysone. A hormone that stimulates the molting process in insects.

Edman degradation. A systematic method of sequencing proteins, proceeding by stepwise removal of single amino acids from the amino terminus of a polypeptide chain.

Eicosanoid. Any fatty acid with 20 carbons.

Electrophoresis. The movement of particles in an electrical field. A commonly-used technique for analysis of mixtures of molecules in solution according to their electrophoretic mobilities.

Elongation factors. Protein factors uniquely required during the elongation phase of protein synthesis. Elongation factor G (EF-G) brings about the movement of the peptidyl tRNA from the A site to the P site of the ribosome.

Eluate. The fluid that has passed through (eluted from) a chromatographic column.

Embryo. Plant or animal at an early stage of development.

Enantiomorphs. Isomers that are mirror images of one another.

Endergonic reaction. A reaction with a positive standard free energy change.

Endocrine glands. Specialized tissues whose function is to synthesize and secrete hormones.

Endonuclease. An enzyme that breaks a phosphodiester linkage at some point within a polynucleotide chain.

Endopeptidase. An enzyme that breaks a polypeptide chain at an internal peptide linkage.

Endoplasmic reticulum. A system of double membranes in the cytoplasm that is involved in the synthesis of transported proteins. The rough endoplasmic reticulum has ribosomes associated with it. The smooth endoplasmic reticulum does not.

End-product (feedback) inhibition. The inhibition of the first enzyme in a pathway by the end product of that pathway.

Energy charge. The fractional degree to which the AMP-ADP-ATP system is filled with high-energy phosphates (phosphoryl groups).

Enhancer. A DNA sequence that can stimulate transcription at an appreciable distance from the site where it is located. It acts in either orientation and either upstream or downstream from the promoter.

Entropy. The randomness of a system.

Enzyme. Complex protein which helps to speed biochemical reactions. Enzymes are important in the construction and degradation of other molecules.or A moleucle, most often a. protein, that contains a catalytic site for a biochemical reaction.

Epimers. Two stereoisomers with more than one chiral center that differ in configuration at one of their chiral centers.

Equilibrium. The point at which the concentrations of two compounds are such that the interconversion of one compound into the other compound does not result in any change in free energy.

Escherichia coli (E. coli). A Gram negative bacterium commonly found in the vertebrate intestine. It is the bacterium most frequently used in the study of biochemistry and genetics.

Established cell line. A group of cultured cells derived from a single origin and capable of stable growth for many generations.

Ether. A molecule containing two carbons linked by an oxygen atom.

Eukaryote. A cell or organism that has a membrane-bound nucleus.

Excision repair. DNA repair in which a damaged region is replaced.

Excited state. An energy-rich state of an atom or a molecule, produced by the absorption of radiant energy.

Exergonic reaction. A chemical reaction that takes place with a negative change in standard free energy.

Exon. A segment within a gene that carries part of the coding information for a protein.

Exonuclease. An enzyme that breaks a phosphodiester linkage at one or the other end of a polynucleotide chain so as to release single or small nucleotide residues.

F factor. A large bacterial plasmid, known as the sex-factor plasmid because it permits mating between F+ and F- bacteria.

Facultative aerobe. An organism that can use molecular oxygen in its metabolism but that also can live anaerobically.

Fatty acid. A long-chain hydrocarbon containing a carboxyl group at one end. Saturated fatty acids have completely saturated hydrocarbon chains. Unsaturated fatty acids have one or more carbon-carbon double bonds in their hydrocarbon chains.

Feedback inhibition. See end-product inhibition.

Fermentation. The energy-generating breakdown of glucose or related molecules by a process that does not require molecular oxygen.

Fingerprinting. The characteristic two-dimensional paper chromatogram obtained from the partial hydrolysis of a protein or a nucleic acid.

Flagellin. Protein which is the primary component of prokaryotic flagella.

Fluorescence. The emission of light by an excited molecule in the process of making the transition from the excited state to the ground state.

Frameshift mutations. Insertions or deletions of genetic material that lead to a shift in the translation of the reading frame. The mutation usually leads to nonfunctional proteins.

Free energy. That part of the energy of a system that is available to do useful work.

Fucoxanthin. Yellowish-brown pigment found in some members of the Chromista, including kelps and diatoms.

Furanose. A sugar that contains a five-membered ring as a result of intramolecular hemiacetal formation.

Futile cycle. See pseudocycle.

G1 phase. That period of the cell cycle in which preparations are being made for chromosome duplication, which takes place in the S phase.

G2 phase. That period of the cell cycle between S phase and mitosis (M phase).

Gametes. The ova and the sperm, haploid cells that unite during fertilization to generate a diploid zygote.

Gel fitration chromatography. A technique that makes use of certain polymers that can form porous beads with varying pore sizes. In columns made from such beads, it is possible to separate molecules, which cannot penetrate beads of a given pore size, from small molecules that can. Also called gel-exclusion or molecular seive chromatography.

Gene amplification. The duplication of a particular gene within a chromosome two or more times.

Gene splicing. The cutting and rejoining of DNA sequences.

Gene. A segment of the genome that codes for a functional product.

General recombination. Recombination that occurs between homologous chromosomes at homologous sites.

Generation time. The time it takes for a cell to double its mass under specified conditions.

Genetic map. The arrangement of genes or other identifiable sequences on a chromosome.

Genome. The total genetic content of a cell or a virus.

Genotype. The genetic characteristics of an organism (distinguished from its observable characteristics, or phenotype).

Globular protein. A folded protein that adopts an approximately globular shape. May also be called soluble proteins.

Gluconeogenesis. The production of sugars from nonsugar precursors such as lactate or amino acids. Applies more specifically to the production of free glucose by vertebrate livers.

Glucose. Simple sugar, and the primary product of photosynthesis. It is polymerized to make cellulose and chitin.

Glycogen. A polymer of glucose residues in 1,4 linkage, with 1,6 linkages at branchpoints.

Glycogenic. Describing amino acids whose metabolism may lead to gluconeogenesis.

Glycolipid. A lipid containing a carbohydrate group.

Glycolysis. The catabolic conversion of glucose to pyruvate with the production of ATP.

Glycoprotein. A membrane-bound protein which has attached branching carbohydrates. These may function in cell-cell recognition, such as in human blood groups and immune system response, as well as in resisting compression of cells.or A protein linked to an oligosaccharide or a polysaccharide. Glycosaminoglycans. Long, unbranched polysaccharide chains composed of repeating disaccharide subunits in which one of the two sugars is either N-acetylglucosamine or N-acetylgalactosamine.

Glycosidic bond. The bond between a sugar and an alcohol. Also the bond that links two sugars in disaccharides, oligosaccharides, and polysaccharides.

Glyoxylate cycle. A pathway that uses some of the enzymes of the TCA cycle and some enzymes whereby acetate can be converted into succinate and carbohydrates.

Glyoxysome. An organelle containing some enzymes of the glyoxylate cycle.

Goldman equation. An equation expressing the quantitative relationship between the concentrations of charged species on either side of a membrane and the resting transmembrane potential.

Golgi apparatus. A complex series of double-membrane structures that interact with the endoplasmic reticulum and that serve as a transfer point for proteins destined for other organelles, the plasma membrane, or extracellular transport.

Gram molecular weight. For a given compound, the weight in grams that is numerically equal to its molecular weight.

Ground state. The lowest electronic energy state of an atom or a molecule.

Growth factor. A substance that must be present in the growth medium to permit eucaryotic cell proliferation.

Growth fork. The region on a DNA duplex molecule where synthesis is taking place. It resembles a fork in shape, since it consists of a region of duplex DNA connected to a region of unwound single strands.

Guanine. A purine base found in DNA or RNA.

Guanosine. A purine nucleoside found in DNA and RNA.

Hairpin loop. A single-stranded complementary region of DNA or RNA that folds back on itself and base-pairs into a double helix.

Half-life. The time required for the disappearance of one half of a substance.

Haploid cell. A cell containing only one chromosome of each type.

Heavy isotopes. Forms of atoms that contain greater numbers of neutrons than the most common form (e.g., ^{15}N, ^{13}C).

Helix. A spiral structure with a repeating pattern.

Heme. An iron-porphyrin complex found in hemoglobin and cytochromes. Hemiacetal. The product formed by the condensation of an aldehyde with an alcohol; it contains one oxygen linked to a central carbon in a hydroxyl fashion and one oxygen linked to the same central carbon by an ether linkage.

Hemoglobin. Protein complex found in the blood of most chordates and the roots of certain legumes. It binds oxgen molecules, and in chordates serves as the means by which the oxygen is supplied to the cells of the body.

Henderson-Hasselbalch equation. An equation that relates the pKa, to the pH and the ratio of the proton acceptor (A^-) and the proton donor (HA) species of a conjugate acid base pair.

Heterochromatin. Highly condensed regions of chromosomes that are not usually transcriptionally active.

Heteroduplex. An annealed duplex structure between two DNA strands that do not show perfect complementarity. Can arise by mutation, recombination, or the annealing of complementary single-stranded DNAs.

Heteropolymer. A polymer containing more than one type of monomeric unit.

Heterotroph. An organism that requires preformed organic compounds for growth.

Heterozygous. Describing an organism (a heterozygote) that carries two different alleles for a given gene.

Hexose. A sugar with a six-carbon backbone.

High-energy compound. A compound that undergoes hydrolysis with a high negative standard free energy change.

Histones. Proteins attached to the DNA of eukaryotes which allows it to be packaged into chromosomes.or The family of basic proteins that is normally associated with DNA in most cells of eukaryotic organisms.

Holoenzyme. An intact enzyme containing all of its subunits and any necessary cofactors with full enzymatic activity.

Homologous chromosomes. Chromosomes that carry the same pattern of genes, but not necessarily the same alleles.

Homopolymer. A polymer composed of only one type of monomeric building block.

Homozygous. Describing an organism (a homozygote) that carries two identical alleles for a given gene.

Hormone receptor. A protein that is located on the cell membrane or inside the responsive cell and that interacts specifically with the hormone.

Hormone. A chemical substance made in one cell and secreted so as to influence the metabolic activity of a select group of cells located at other sites in the organism.

Host cell. A cell used for growth and reproduction of a virus.

Hybrid (or chimeric) plasmid. A plasmid that contains DNA from two different organisms.

Hydrogen bond. A weak, noncovalent, attractive force between one electronegative atom and a hydrogen atom that is covalently linked to a second electronegative atom.

Hydrolysis. The cleavage of a molecule by the addition of water. Hydrophilic. Preferring to be in contact with water.

Hydrophilic. "Water loving". Hydrophilic compounds dissolve easily in water, and are usually polar.

Hydrophobic effect. The noncovalent association of nonpolar groups with each other in aqueous solution.

Hydrophobic. "Water fearing". Hydrophobic compounds do not dissolve easily in water, and are usually non-polar. Oils and other long hydrocarbons are hydrophobic.or Preferring not to be in contact with water, as is the case with the hydrocarbon portion of a fatty acid or phospholipid chain.

Hydroxyapatite. A calcium phosphate gel used, in the case of nucleic acids, to selectively absorb duplex DNA-RNA from a mixture of single-stranded and duplex nucleic acids.

Icosahedral symmetry. The symmetry displayed by a regular polyhedron that is composed of 20 equilateral triangular faces with 12 corners.

Imine. A molecule containing a nitrogen atom attached to a carbon atom by a double bond. The nitrogen is also covalently linked to a hydrogen. Immunofluorescence. A cytological technique in which a specific fluorescent antibody is used to label an antigen. Frequently used to determine the location of an antigen in a tissue or a cell.

Immunoglobulin. A protein made in a B plasma cell and usually secreted; it interacts specifically with a foreign agent. Synonymous with antibody. It is composed of two heavy and two light chains linked by disulfide bonds. Immunoglobulins can be divided into five classes (IgG, IgM, IgA, IgD, and IgE) based on their heavy-chain component.

In vitro. Literally, "in glass," describing whatever happens in a test tube or other receptacle, as opposed to what happens in whole cells of the whole organism (*in vivo*).

Induced fit. A change in the shape of an enzyme that results from the binding of substrate.

Inducers. Molecules that cause an increase in a protein activity when added to cells.

Inducible proteins. Those which are synthesized in different amounts depending on cellular signals.

Initiation factors. Those protein factors that are specifically required during the initiation phase of protein synthesis.

Integrin. Adhesive protein of the extracellular matrix in animals.

Intercalating agent. A chemical, usually containing aromatic rings, that can sandwich in-between adjacent base pairs in a DNA duplex. The intercalation leads to an adjustment in the DNA secondary structure, as adjacent base pairs are usually close-packed.

Interferon. One of a family of proteins that are liberated by special host cells in the mammal in response to viral infection. The interferons attach to an infected cell, where they stimulate antiviral protein synthesis.

Intervening sequence. See intron.

Intron. A segment of the nascent transcript that is removed by splicing. Also refers to the corresponding region in the DNA. Synonymous with intervening sequence.

Inverted repeat. A chromosome segment that is identical to another segment on the same chromosome except that it is oriented in the opposite direction.

Ion. An atom or small molecule which carries a positive or negative charge.

Ion-exchange resin. A polymeric resinous substance, usually in bead form, that contains fixed groups with positive or negative charge. An anion exchange resin has positively-charged groups and is therefore useful in exchanging the anionic groups in a test sample; a cation exchange resin is itself negatively charged, and has the opposite application. The resin is usually used in a column chromatographic procedure.

Isoelectric point or pH. The pH at which a protein has no net charge.

Isomerase. An enzyme that catalyzes an intramolecular rearrangement.

Isomerization. Rearrangement of atomic groups within the same molecule without any loss or gain of atoms.

Isozymes. Multiple forms of an enzyme that differ from one another in one or more of the properties.

Ketogenic. Describing amino acids that are metabolized to acetoacetate and acetate.

Ketone bodies. Refers to acetoacetate, acetone, and □-hydroxybutyrate made from acetyl-CoA in the liver and used for energy in nonhepatic tissue.

Ketone. A functional group of an organic compound in which a carbon atom is double-bonded to an oxygen. Neither of the other substituents attached to the carbon is a hydrogen. Otherwise the group would be called an aldehyde.

Ketosis. A condition in which the concentration of ketone bodies in the blood or urine is unusually high.

Kilobase. One thousand bases in a DNA molecule.

Kinase. An enzyme catalyzing phosphorylation of an acceptor molecule, usually with ATP serving as the phosphate (phosphoryl) donor.

Kinetochore. A structure that attaches laterally to the centromere of a chromosome; it is the site of chromosome tubule attachment.

Km. See Michaelis constant.

Krebs cycle. See tricarboxylic acid (TCA) cycle.

Laminarin. A beta-glucan polysaccharide produced by many chromists through photosynthesis.

Lampbrush chromosome. Giant diplotene chromosome found in the oocyte nucleus. The loops that are observed are the sites of extensive gene expression.

Law of mass action. The finding that the rate of a chemical reaction is a function of the product of the concentrations of the reacting species.

Leader region. The region of an mRNA between the 5' end and the initiation codon for translation of the first polypeptide chain.

Leader sequence. An N-terminal signal sequence that directs secretion and processing of proteins.

Lectins. Agglutinating proteins usually extracted from plants.

Ligand. A (usually small) molecule that binds to another, such as oxygen when it binds to myoglobin.

Ligase. An enzyme that catalyzes the joining of two molecules together. In DNA it joins 5'-OH to 3' phosphates.

Linkage. The tendency of markers to be inherited together. Linkage of two markers is an indication that they are close to one another in the genome.

Linkers. Short oligonucleotides that can be ligated (connected) to larger DNA fragments, then cleaved (cut) to yield overlapping cohesive (sticky) ends, suitable for ligation to other DNAs that contain comparable cohesive ends.

Linking number. The net number of times one polynucleotide chain crosses over another polynucleotide chain. By convention, right-handed crossovers are given a plus designation.

Lipid bilayer. Model for the structure of the cell membrane based on the interaction between the hydrophobic regions of phospholipids.

Lipid. A biological molecule that is soluble in organic solvents. Lipids include steroids, fatty acids, prostaglandins, terpenes, and waxes. or A class of biochemical compounds which includes fats, oils, and waxes.

Lipopolysaccharide. Usually refers to a unique glycolipid found in Gram negative bacteria.

Luciferase. Enzyme which activates luciferin to produce bioluminescence.

Luciferin. Compound whose activated form emits light.

Lyase. An enzyme that catalyzes the removal of a group to form a double bond, or the reverse reaction.

Lysogenic virus. A virus that can adopt an inactive (lysogenic) state, in which it maintains its genome within a cell instead of entering the lytic cycle. The circumstances that determine whether a lysogenic (temperate) virus will adopt an inactive state or an active lytic state are often subtle and depend upon the physiologic state of the infected cell.

Lysosome. An organelle that contains hydrolytic enzymes designed to break down proteins that are targeted to that organelle.

Lytic infection. A virus infection that leads to the lysis of the host cell, yielding progeny virus particles.

M phase. That period of the cell cycle when mitosis takes place.

Marker. A "landmark" that can be localized to a specific region of the genome.

Meiosis. Process in which diploid cells undergo division to form haploid sex cells.

Membrane protein. A protein that is associated with a membrane, rather than found free in the cell. A membrane protein may be integral (embedded or buried) in the membrane, or peripheral (attached more loosely, by interactions with either lipid or intergral membrane proteins).

Membrane transport. The facilitated transport of a molecule across a membrane.

Membrane. A sheet-like composite of protein and lipid that is the boundary of cells and organelles.

Merodiploid. An organism that is diploid for some but not all of its genes.

Mesosome. An invagination of the bacterial cell membrane.

Messenger RNA (mRNA). The template RNA carrying the message for protein synthesis.

Metabolic turnover. A measure of the rate at which already existing molecules of the given species are replaced by newly-synthesized molecules of the same type. Usually isotopic labeling is required to measure turnover.

Metabolism. The sum total of the enzyme-catalyzed reactions that occur in a living organism.

Metamorphosis. A change of form, especially the conversion of a larval form to an adult form.

Metaphase. That stage in mitosis or meiosis when all of the chromosomes are lined up on the equator (i.e., an imaginary line that bisects the cell).

Micelle. An aggregate of lipids in which the polar head groups face outward and the hydrophobic tails face inward; no solvent is trapped in the center.

Michaelis constant (Km). The substrate concentration at which an enzyme-catalyzed reaction proceeds at one-half of the maximum velocity.

Michaelis-Menten equation Or Henri-Michaelis-Menten equation. An equation relating the reaction velocity to the substrate concentration of an enzyme.

Microtubules. Thin tubules, made from globular proteins, that serve multiple purposes in eukaryotic cells.

Mismatch repair. The replacement of a base in a heteroduplex structure by one that forms a Watson-Crick base pair.

Missense mutation. A change in which a codon for one amino acid is replaced by a codon for another amino acid.

Mitochondrion. An organelle, found in eukaryotic cells, in which oxidative phosphorylation takes place. It contains its own genome and unique ribosomes to carry out protein syn thesis of only a fraction of the proteins located in this organelle.

Mitosis. The process whereby replicated chromosomes segregate equally toward opposite poles prior to cell division.

Mobile genetic element. A segment of the genome that can move as a unit from one location on the genome to another, without any requirement for sequence homology.

Molecular seive chromatography. See Gel filtration chromatography.

Molecular weight. See Gram molecular weight.

Molecularity of a reaction. The number of molecules involved in a specific reaction step.

Monolayer. A single layer of oriented lipid molecules.

Monomer. One unit of a protein or other structure.

Mutagen. An agent that can bring about a heritable change (mutation) in an organism.

Mutagenesis. A process that leads to a change in the genetic material that is inherited in later generations.

Mutant. An organism that carries an altered gene or change in its genome.

Mutarotation. The change in optical rotation of a sugar that is observed immediately after it is dissolved in aqueous solution, as the result of the slow approach of equilibrium of a pyranose or a furanose in its alpha and beta forms.

Mutation. The genetically inheritable alteration of a gene or group of genes.

Myofibril. A unit of thick and thin filaments in a muscle fiber.

Myosin. The main protein of the thick filaments in a muscle myofibril. It is composed of two coiled subunits (M_r about 220,000) that can aggregate to form a thick filament, which is globular at each end.

Nascent RNA. The initial transcripts of RNA, before any modification or processing.

Negative control. Repression of biological activity by the presence of a specific molecule.

Nernst equation. An equation that relates the redox potential to the standard redox potential and the concentrations of the oxidized and reduced form of the couple.

Neurotoxin. Poison which interferes with nerve function, usually by affecting the flow of ions through the cell membrane.

Nitrogen cycle. The passage of nitrogen through various valence states, as the result of reactions carried out by a wide variety of different organisms.

Nitrogen fixation. Conversion of atmospheric nitrogen into a form that can be converted by biochemical reactions to an organic form. This reaction is carried out by a very limited number of microorganisms.

Nitrogenous base. An aromatic nitrogen-containing molecule with basic properties. Such bases include purines and pyrimidines.

Noncompetitive inhibitor. An inhibitor of enzyme activity whose effect is not reversed by increasing the concentration of substrate molecule.

Nonsense mutation. A change in the base sequence that converts a sense codon (one that specifies an amino acid) to one that specifies a stop (a nonsense codon). There are three nonsense codons: amber, ochre and something I forget (let me know if you read this - S. Mowbray).

Northern blotting. See Southern blotting.

Nuclease. An enzyme that cleaves phosphodiester bonds of nucleic acids.

Nucleic Acid. Class of biochemical compounds which includes DNA and RNA. They are among the largest molecules known. or Polymers of the ribonucleotides or deoxyribonucleotides.

Nucleic acids.

Nucleohistone. A complex of DNA and histone.

Nucleolus. A spherical structure visible in the nucleus during interphase. The nucleolus is associated with a site on the chromosome that is involved in ribosomal RNA synthesis.

Nucleophilic group. An electron-rich group that tends to attack an electron-deficient nucleus.

Nucleoside. An organic molecule containing a purine or pyrimidine base and a five-carbon sugar (ribose or deoxyribose).

Nucleosome. A complex of DNA and an octamer of histone proteins in which a small stretch of the duplex is wrapped around a molecular bead of histone.

Nucleotide. An organic molecule containing a purine or pyrimidine base, a five-carbon sugar (ribose or deoxyribose), and one or more phosphate groups. A phosphoester of a nucleoside. or Unit from which nucleic acids are constructed by polymerization. It contains a sugar, a phosphate group, and an organic base. Atp is a nucleotide.

Nucleotide.

Nucleus. In eukaryotic cells, the centrally-located organelle that encloses most of the chromosomes. Minor amounts of chromosomal substance are found in some other organelles, most notably the mitochondria and the chloroplasts.

Okazaki fragment. A short segment of single-stranded DNA that is an intermediate in DNA synthesis. In bacteria, Okazaki fragments are 1000-2000 bases in length; in eukaryotes, 100-200 bases in length.

Oligonucleotide. A polynucleotide containing a small number of nucleotides. The linkages are the same as in a polynucleotide; the only distinguishing feature is the small size.

Oligosaccharide. A molecule containing a small number of sugar residues joined in a linear or a branched structure by glycosidic bonds.

Oncogene. A gene of cellular or viral origin that is responsible for rapid, unruly growth of animal cells. A cancer-causing gene.

Operon. A group of contiguous genes that are coordinately regulated by two cis-acting elements, a promoter and an operator. Found only in prokaryotic cells.

Optical activity. The property of a molecule that leads to rotation of the plane of polarization of plane-polarized light when the latter is transmitted through the substance. Chirality is a necessary and sufficient property for optical activity.

Organelle. A subcellular membrane-bounded body with a well-defined function.

Osmotic pressure. The pressure generated by the mass flow of water to that side of a membrane-bounded structure that contains the higher concentration of solute molecules. A stable osmotic pressure is seen in systems in which the membrane is not permeable to some of the solute molecules.

Oxidation. The loss of electrons from a compound.

Oxidative phosphorylation. The formation of ATP as the result of the transfer of electrons to oxygen.

Oxido-reductase. An enzyme that catalyzes oxidation-reduction reactions.

Palindrome. A sequence of bases that reads the same in both directions on opposite strands of the DNA duplex (e.g., GAATTC).

PCR. Polymerase chain reaction. A method for amplifying DNA sequences.

Pentose phosphate pathway. The pathway involving the oxidation of glucose-6-phosphate to pentose phosphates and further reactions of pentose phosphates.

Pentose. A sugar with five carbon atoms.

Peptide mapping. Same as fingerprinting.

Peptide. An organic molecule in which a covalent amide bond is formed between the α-amino group of one amino acid and the α-carboxyl group of another amino acid, with the elimination of a water molecule. The resulting connection is called a peptide bond.

Peptidoglycan. The main component of the bacterial cell wall, consisting of a two-dimensional network of heteropolysaccharides running in one direction, cross-linked with polypeptides running in the perpendicular direction. or Carbohydrate polymer cross-linked by proteins. It is found in the cell wall of gram positive bacteria, where it stains with the dye crystal-violet.

Peridinin. Carotenoid pigment found in dinoflagellates.

Periplasm. The region between the inner (cytoplasmic) membrane and the cell wall or outer membrane of a bacterium.

Permeable. The property of allowing material to pass through, as a permeable membrane.

Permease. A protein that catalyzes the transport of a specific small molecule across a membrane.

Peroxisomes. Subcellular organelles that contain flavin-requiring oxidases and that regenerate oxidized flavin by reaction with oxygen.

Phenotype. The observable trait(s) that result from the genotype in cooperation with the environment.

Phenylketonuria. A human disease caused by a genetic deficiency in the enzyme that converts phenylalanine to tyrosine. The immediate cause of the disease is an excess of phenylalanine, which can be alleviated by a diet low in phenylalanine.

Pheromone. A hormone-like substance that acts as an attractant.

Phosphate. An ion consisting of a phosphorus atom and four oxygen atoms. Among other things, it is used in the constuction of nucleic acids.

Phosphodiester. A molecule containing two alcohols esterified to a single molecule of phosphate. For example, the backbone of nucleic acids is connected by 5'-3' phosphodiester linkages between the adjacent individual nucleotide residues.

Phosphogluconate pathway. Another name for the pentose phosphate pathway. This name derives from the fact that 6-phosphogluconate is an intermediate in the formation of pentoses from glucose.

Phospholipid. A lipid containing charged hydrophilic phosphate groups; a component of cell membranes.

Phosphorylation. The formation of a phosphate derivative of a biomolecule.

Photoreactivation. DNA repair in which the damaged region is repaired with the help of light and an enzyme. The lesion (break) is repaired without excision (cutting out) from the DNA.

Photosynthesis. The biosynthesis that directly harnesses the chemical energy resulting from the absorption of light. Frequently used to refer to the formation of carbohydrates from CO_2 that occurs in the chloroplasts of plants or the plastids of photosynthetic microorganisms. or Biochemical process in which light energy is absorbed by chlorophyll, and is used to fuel the building of sugar molecules.

Phycocyanin. Blue, water-soluble pigment found in the cyanobacteria and the red algae.

Phycoerythrin. Red, water-soluble pigment found in the cyanobacteria and red algae.

Pigment. Any colorful compound, used by living things to absorb or block sunlight, and in sexual displays.

Pitch length (or pitch). The number of base pairs per turn of a duplex helix.

Plaque. A circular clearing on a lawn (continuous layer) of bacterial or culture cells, resulting from cell Iysis and production of phage or animal virus progeny.

Plasma membrane. The membrane that surrounds the cytoplasm.

Plasmid. A circular DNA duplex that replicates autonomously in bacteria. Plasmids that integrate into the host genome are called episomes. Plasmids differ from viruses in that they never form infectious nucleoprotein particles.

Polar group. A hydrophilic (water-loving) group.

Polar mutation. A mutation in one gene that reduces the expression of a gene or genes distal to the promoter in the same operon.

Polarimeter. An instrument for determining the rotation of polarization of light as the light passes through a solution containing an optically-active substance.

Polyamine. A hydrocarbon containing more than two amino groups.

Polycistronic messenger RNA. In prokaryotes, an RNA that contains two or more cistrons; note that only in prokaryotic mRNAs can more than one cistron be utilized by the translation system to generate individual proteins.

Polymer. A large molecule constructed from many smaller identical units. These include proteins, nucleic acids, and starches.

Polymerase. An enzyme that catalyzes the synthesis of a polymer from monomers.

Polynucleotide phosphorylase. An enzyme that polymerizes ribonucleotide diphosphates. No template is required.

Polynucleotide. A chain structure containing nucleotides linked together by phosphodiester (5'-3') bonds. The polynucleotide chain has a directional sense with a 5' and a 3' end.

Polypeptide. A linear polymer of amino acids held together by peptide linkages. The polypeptide has a directional sense, with an amino- and a carboxy-terminal end.

Polyribosome (polysome). A complex of an mRNA and two or more ribosomes actively engaged in protein synthesis.

Polysaccharide. A linear or branched chain structure containing many sugar molecules linked by glycosidic bonds.

Porphyrin. A complex planar structure containing four substituted pyrroles covalently joined in a ring and frequently containing a central metal atom. For example, heme is a porphyrin with a central iron atom.

Positive control. A system that is turned on by the presence of a regulatory protein.

Posttranslational modification. The covalent bond changes that occur in a polypeptide chain after it leaves the ribosome and before it becomes a mature protein.

Primary structure. In a polymer, the sequence of monomers and the covalent bonds. In proteins, it refers to the amino acid sequence.

Primer. A structure that serves as a growing point for polymerization. Short primers of DNA are often used in sequencing and mutagenesis procedures.

Primosome. A multiprotein complex that catalyzes synthesis of RNA primer at various points along the DNA template.

Prochiral molecule. A nonchiral molecule that lacks handedness and is optically inactive, but would become chiral by a change in one of the substituents at the chiral center. A prochiral molecule may react with an enzyme so that two groups that have a mirror-image relationship to each other are treated differently.

Prokaryote. A unicellular organism that contains a single chromosome, no nucleus, no membrane-bound organelles, and has characteristic ribosomes and biochemistry.

Promoter. That region of the gene that signals RNA polymerase binding and the initiation of transcription.

Prophage. The silent phage genome. Some prophages integrate into the host genome; others replicate autonomously. The prophage state is maintained by a phage-encoded repressor.

Prophase. The stage in meiosis or mitosis when chromosomes condense and become visible as refractile bodies.

Proprotein. A protein that is made in an active form, so that it requires processing to become functional.

Prostaglandin. An oxygenated eicosanoid that has a hormonal function. Prostaglandins are unusual hormones in that they usually have effects only in that region of the organism where they are synthesized.

Prosthetic group. Synonymous with coenzyme except that a prosthetic group is usually more firmly attached to the enzyme it serves.

Protamines. Highly basic, arginine-rich proteins found complexed to DNA in the sperm of many invertebrates and fish.

Protein subunit. One of the components or monomers of a multicomponent protein.

Protein. Class of biochemical compounds constructed from amino acids. Proteins may be structural, such as those that make up hair and cartilage, or they may be reactive, such as the enzymes.

Proteinaceous. Describes any structure which is composed of protein.

Proteoglycan. A protein-linked heteropolysaccharide in which the heteropolysaccharide is usually the major component.

Protist. A relatively undifferentiated organism that can survive as a single cell.

Proton acceptor. A functional group capable of accepting a proton from a proton donor molecule.

Proton motive force (□p). The thermodynamic driving force for proton translocation.

Proto-oncogene. A cellular gene that can undergo modification to a cancer-causing gene (oncogene).

Pseudocycle. A sequence of reactions that can be arranged in a cycle but that usually do not function simultaneously in both directions. Also called a futile cycle, since the net result of simultaneous functioning in both directions would be the expenditure of energy without accomplishing any useful work.

Pulse-chase. An experiment in which a short labeling period is followed by the addition of an excess of the same, unlabeled compound to dilute out the labeled material. Useful for observing time-dependent behavior of compounds.

Purine. A heterocyclic ring structure with varying functional groups. The purines adenine and guanine are found in both DNA and RNA.

Puromycin. An antibiotic that inhibits polypeptide synthesis by competing with aminoacyl-tRNA for the ribosomal binding site A.

Pyranose. A simple sugar containing the six-membered pyran ring.

Pyrimidine. A heterocyclic six-membered ring structure. Cytosine and uracil are the main pyrimidines found in RNA, and cytosine and thymine are the main pyrimidines found in DNA.

Pyrophosphate. A molecule formed by two phosphates in anhydride linkage.

Quaternary structure. In a protein, the way in which the different folded subunits interact to form the multisubunit protein.

R group. Shorthand for the side chain of an amino acid.

R loop. A triple-stranded structure in which RNA displaces a DNA strand by DNA-RNA hybrid formation in a region of the DNA.

Rapid-start complex. The complex that RNA polymerase forms at the promoter site just before initiation.

rbcL. A gene which is located in the chloroplast of photosynthetic organisms. It codes for the large subunit of the protein rubisco, and its sequence has been useful in plant phylogenies.

Recombination. The transfer to offspring of genes not found together in either of the parents.

Redox couple. An electron donor and its corresponding oxidized form.

Redox potential (E). The relative tendency of a pair of molecules to release or accept an electron. The standard redox potential (E^0) is the redox potential of a solution containing the oxidant and reductant of the couple at standard concentrations.

Regulatory enzyme. An enzyme in which the active site is subject to regulation by factors other than the enzyme substrate. The enzyme frequently contains a nonoverlapping site for binding the regulatory factor that affects the activity of the active site.

Regulatory gene. A gene whose principal product is a protein designed to regulate the synthesis of other genes.

Renaturation. The process of returning a denatured structure to its original native structure, as when two single strands of DNA are reunited to form a regular duplex, or an unfolded polypeptide chain is returned to its normal folded three-dimensional structure.

Repair synthesis. DNA synthesis following excision (cutting out) of damaged DNA.

Repetitive DNA. A DNA sequence that is present in many copies per genome.

Replica plating. A technique in which an impression of a culture is taken from a master plate and transferred to a fresh plate. The impression can be of bacterial clones or phage plaques.

Replication fork. The Y-shaped region of DNA at the site of DNA synthesis; also called a growth fork.

Replicon. A genetic element that behaves as an autonomous replicating unit. It can be a plasmid, phage, or bacterial chromosome.

Repressor. A regulatory protein that inhibits transcription from one or more genes. It can combine with an inducer (resulting in specific enzyme induction) or with an operator element (resulting in repression).

Resonance hybrid. A molecular structure that is a hybrid of two structures that differ in the locations of some of the electrons. For example, the benzene ring can be drawn in two ways, with double bonds in different positions. The actual structure of benzene is in-between these two equivalent structures.

Restriction-modification system. A pair of enzymes found in most bacteria (but not eukaryotic cells). The restriction enzyme recognizes a certain sequence in duplex DNA and makes one cut in each unmodified DNA strand at or near the recognition sequence. The modification enzyme methylates (or modifies) the same sequence, thus protecting it from the action of the restriction enzyme.

Reverse transcriptase. An enzyme that synthesizes DNA from an RNA template, using deoxyribonucleotide triphosphates.

Rho factor. A protein involved in the termination of transcription of some messenger RNAs.

Ribose. The five-carbon sugar found in RNA.

Ribosomal RNA (rRNA). The RNA parts of the ribosome.

Ribosomes. Small cellular particles made up of ribosomal RNA and protein. They are the site, together with mRNA, of protein synthesis.

RNA (ribonucleic acid). A polynucleotide in which the sugar is ribose.

RNA polymerase. An enzyme that catalyzes the formation of RNA from ribonucleotide triphosphates, using DNA as a template.

RNA splicing. The excision of a segment of RNA, followed by a rejoining of the remaining fragments.

RNA. "Ribonucleic acid". The nucleic acid which carries the dna message into parts of the cell where it is interpreted and used. The 18s ribosomal rna sequence has been used in many groups of organisms to reconstruct phylogeny.

Rolling circle replication. A mechanism for the replication of circular DNA. A nick in one strand allows the 3' end to be extended, displacing the strand with the 5' end, which is also replicated, to generate a double- stranded tail that can become larger than the unit size of the circular DNA.

Rubisco. Protein which fixes carbon in photosynthetic organisms. It binds molecules of carbon dioxide to a five-carbon molcule. Rubisco is the most common protein on earth.

S phase. The period during the cell cycle when the chromosome is replicated.

Salting in. The increase in solubility that is displayed by typical globular proteins upon the addition of small amounts of certain salts, such as ammonium sulfate.

Salting out. The decrease in protein solubility that occurs when salts such as ammonium sulfate are present at high concentrations.

Salvage pathway. A family of reactions that permits, for instance, nucleosides as well as purine and pyrimidine bases resulting from the partial breakdown of nucleic acids to be re-utilized in nucleic acid synthesis.

Satellite DNA. A DNA fraction whose base composition differs from that of the main component of DNA, as revealed by the fact that it bands at a different density in a CsCI gradient. Usually repetitive DNA or organelle DNA.

Saxitoxin. Neurotoxin found in a variety of dinoflagellates. If ingested, it may cause respiratory failure and cardiac arrest.

Second messenger. A diffusible small molecule, such as cAMP, that is formed at the inner surface of the plasma membrane in response to a hormonal signal.

Secondary structure. In a protein or a nucleic acid, any repetitive folded pattern that results from the interaction of the corresponding polymeric chains. In proteins, the most common are [illegible] -strands (sheets) and α-helices.

Semiconservative replication. Duplication of DNA in which the daughter duplex carries one old strand and one new strand.

Semipermeable. The characteristic of allowing only some molecules, usually smaller or uncharged ones, to pass through.

Sigma factor. A subunit of RNA polymerase that recognizes specific sites on DNA for initiation of RNA synthesis.

Signal sequence. A (usually N-terminal) sequence of a protein that directs its processing or localization within the cell.

Silica. Amorphous silicon dioxide (glass). It is a structural component in many organisms, such as diatoms and horsetails.

Single-copy DNA. A region of the genome whose sequence is present only once per haploid complement.

Site-directed mutagenesis. An intentional alteration in a DNA sequence that can be genetically inherited.

Soluble protein. See globular protein.

Somatic cell. Any cell of an organism that cannot contribute its genes to a later generation.

SOS system. A set of DNA repair enzymes and regulatory proteins that regulate their synthesis so that maximum syn thesis occurs when the DNA is damaged.

Southern blotting. A method for detecting a specific DNA restriction fragment, developed by Edward Southern. DNA from a gel electrophoresis pattern is blotted onto nitrocellulose paper; then the DNA is denatured and fixed on the paper. Subsequently the pattern of specific sequences in the Southern blot can be determined by hybridization to a suitable probe and autoradiography. A Northern blot is similar, except that RNA is blotted instead onto the nitrocellulose paper.

Splicing. See RNA splicing.

Spongin. Proteinacous compound of which the spicules in Demospongiae are composed.

Sporulation. Formation from vegetative cells of metabolically inactive cells that can resist extreme environmental conditions.

Stacking energy. The energy of interaction that favors the face-to-face packing of purine and pyrimidine base pairs.

Starch. A complex polymer of glucose, used by plants and green algae to store surplus sugar for later use.

Steady state. In enzyme-kinetic analysis, the time interval when the rate of reaction is approximately constant with time. The term is also used to describe the state of a living cell where the concentrations of many molecules are approximately constant because of a balancing between their rate of synthesis and breakdown.

Stem cell. A cell from which other cells stem or arise by differentiation.

Stereoisomers. Isomers that are nonsuperimposable mirror images of each other.

Steroids. Compounds that are derivatives of a tetracyclic structure composed of a cyclopentane ring fused to a substituted phenanthrene nucleus.

Structural domain. An element of protein tertiary structure that forms an independent folding unit.

Structural gene. A gene encoding the amino acid sequence of a polypeptide chain.

Structural protein. A protein that serves a structural function.

Substrate. A molecule that is acted upon, and chemically changed, by an enzyme.

Subunit. Individual polypeptide chains in a protein.

Sugar. Any of several small carbohydrates, such as glucose, which are "sweet" to the taste.

Supercoiled DNA. Supertwisted, covalently-closed duplex DNA.

Suppressor gene. A gene that can reverse the phenotype of a mutation in another gene.

Suppressor mutation. A mutation that restores a function lost by an initial mutation and that is located at a site different from the initial mutation.

Svedberg unit (S). The unit used to express the sedimentation constant ($S = 10^{-13}$ sec). The sedimentation constant S is proportional to the rate of sedimentation of a molecule in a given centrifugal field and is related to the size and shape of the molecule.

Synapse. The chemical connection for communication between two nerve cells or between a nerve cell and a target cell such as a muscle cell.

Synapsis. The pairing of homologous chromosomes, seen during the first meiotic prophase.

Tandem duplication. A duplication in which the repeated regions are immediately adjacent to one another.

TCA cycle. See tricarboxylic acid cycle.

Template. A polynucleotide chain that serves as a surface for the absorption of monomers of a growing polymer and thereby dictates the sequence of the monomers in the growing chain.

Termination factors. Proteins that are exclusively involved in the termination reactions of protein synthesis on the ribosome.

Terpenes. A diverse group of lipids made from isoprene precursors.

Tertiary structure. In a protein or nucleic acid, the final folded form of the polymer chain.

Tetramer. Structure resulting from the association of four subunits.

Thioester. An ester of a carboxylic acid with a thiol or mercaptan.

Thymidine. One of the four nucleosides found in DNA.

Thymine. A pyrimidine base found in DNA.

Topoisomerase. An enzyme that changes the extent of supercoiling of a DNA duplex.

Transamination. Enzymatic transfer of an amino group from an α-amino acid to an α-keto acid.

Transcription. RNA synthesis that occurs on a DNA template.

Transduction. Genetic exchange in bacteria that is mediated via phage.

Transfection. An artificial process of infecting cells with naked viral DNA.

Transfer RNA (tRNA). Any of a family of low-molecular weight RNAs that transfer amino acids from the cytoplasm to the template for protein synthesis on the ribosome.

Transferase. An enzyme that catalyzes the transfer of a molecular group from one molecule to another.

Transformation. Genetic exchange in bacteria that is mediated via purified DNA. In somatic cell genetics the term is also used to indicate the conversion of a normal cell to one that grows like a cancer cell.

Transgenic. Describing an organism that contains transfected DNA in the germ line.

Transition state. The activated state in which a molecule is best suited to undergoing a chemical reaction.

Translation. The process of reading a messenger RNA sequence for the specified amino acid sequence it contains.

Transport protein. A protein whose primary function is to transport a substance from one part of the cell to another, from one cell to another, or from one tissue to another.

Tricarboxylic acid (TCA) cycle. The cyclical process whereby acetate is completely oxidized to CO2 and water, and electrons are transferred to NAD^+ and flavine. The TCA cycle is localized to the mitochondria in eukaryotic cells and to the plasma membrane in prokaryotic cells. Also called the Krebs or citric acid cycle.

Trypsin. A proteolytic enzyme that cleaves (cuts) peptide chains next to the basic amino acids arginine and Iysine.

Tryptic peptide mapping. The technique of generating a chromatographic profile characteristic of the fragments resulting from trypsin enzyme cleavage of the protein.

Tumorigenesis. The mechanism of tumor formation.

Turnover number. The maximum number of molecules of substrate that can be converted to product per active site per unit time.

Ultracentrifuge. A high-speed centrifuge that can attain speeds up to 60,000 rpm and centrifugal fields of 500,000 times gravity. Useful for characterizing and/or separating macromolecules.

Unidirectional replication. See bidirectional replication.

Unwinding proteins. Proteins that help to unwind double-stranded DNA during DNA replication.

Urea cycle. A metabolic pathway in the liver that leads to the synthesis of urea from amino groups and CO_2. The function of the pathway is to convert the ammonia resulting from catabolism to a nontoxic form, which is then secreted.

UV irradiation. Electromagnetic radiation with a wavelength shorter than that of visible light (200-390 nm). Causes damage to DNA (mainly by forming pyrimidine dimers).

van der Waals forces. Refers to the combined effect of two types of interactions, one attractive and one repulsive. The attractive forces are due to favorable interactions among the induced instantaneous dipole moments that arise from fluctuations in the electron charge densities of neighboring nonbonded atoms. Repulsive forces arise when noncovalently bonded atoms come too close together.

Viroids. Pathogenic agents, mostly of plants, that consist of short (usually circular) RNA molecules.

Virus. A complex of nucleic-acid and protein, that can infect and replicate inside a specific host cell to make more virus particles.

Vitamin. A trace organic substance required in the diet of some species. Many vitamins are precursors of coenzymes.

Watson-Crick base pairs. The type of hydrogen-bonded base pairs found in DNA, or comparable base pairs found in RNA. The base pairs are A-T, G-C, and A-U.

Western blot. Similar in principle to a Southern blot, but where the species adsorbed to the nitrocellulose filter is a protein, and the detection makes use of specific antibodies.

Wild-type gene. The form of a gene (allele) normally found in nature.

Wobble. A proposed explanation for base pairing that is not of the Watson-Crick type and that often occurs between the 3' base in the codon and the 5' base in the anticodon.

X-ray crystallography. A technique for determining the structure of molecules from the X-ray diffraction patterns that are produced by crystalline arrays of the molecules.

Ylid. A compound in which adjacent, covalently-bonded atoms, both having an electronic octet, have opposite charges.

Z form. A duplex DNA structure in which there is the usual type of hydrogen bonding between the base pairs but in which the helix formed by the two polynucleotide chains is left-handed rather than right-handed.

Zwitterion. A dipolar ion with spatially-separated positive and negative charges. For example, most amino acids are zwitterions, having a positive charge on the α-amino group and a negative charge on the α-carboxyl group but no net charge on the overall molecule.

Zygote. A cell that results from the union of haploid male and female sex cells. Zygotes are diploid.

Zymogen. An inactive precursor of an enzyme. For example, trypsin exists in the inactive form trypsinogen before it is converted to its active form, trypsin.

Suggested Readings

- Berg JM, Tymoczko JL and Stryer L 2002. Biochemistry, 5th edition, W H Freeman & Company, England.
- Devlin TM 2001. Textbook of Biochemistry with Clinical Correlations Fifth edition, John Wiley & Sons, Inc. Publication, New York.
- Garrett RH and Grisham CM 1999. Biochemistry. Second edition, Saunders College Publishing, New York.
- Goodwin TW and Mercer EI 1983. Introduction to Plant Biochemistry, Second edition, Pergamon Press, New York.
- Harborne JB and Dey PM 1997. Plant Biochemistry, First edition, Academic Press, San Diego.
- Hames BD and Hooper NM 2003. Instant Notes Biochemistry, Third edition, Viva Books Private Limites, New Delhi.
- Karp G 1999. Cell and Molecular Biology, 2nd Edition, John Wiley & Sons, Inc., USA.
- Lehninger AL, Nelson DL and Cox MM 1993. Principles of Biochemistry, CBS Publishers & Distributors, Delhi.
- Mann APS, Munshi SK, Gupta AK 2001. Biochemistry - Environment and Agriculture, Xpress Graphics, Delhi.
- Murray RK, Granner DK, Mayes PA and Rodwell VW 2000. Harper's Biochemistry, Twenty fifth editions, Prentice-Hall International, New York.
- Sawhney SK and Randhir Singh 2000. Introductory Practical Biochemistry, Narosa Publishing House Ltd, New Delhi.
- Voet D and Voet JG 2001. Biochemistry. Third edition, John Wiley & Sons, New York.
- Well JH (2004). General Biochemistry, 6th Edition, New Age International (P) Ltd., New delhi.

“This Page is Intentionally Left Blank”

Common Important Abbreviations

A : Adenine
Ab : Antibody
ACAT : Acyl-coa:cholesterol acyl transferase
Ac-CoA : Acetyl-coenzyme A
ACh : Acetylcholine
ACP : Acyl carrier protein
ADH : Alcohol dehydrogenase
AdoMet : *S*-adenosylmethionine
ADP : Adenosine diphosphate
Ag : Antigen
AIDS : Acquired immune deficiency syndrome
Ala : Alanine
ALA : δ-Aminolevulinic acid
Ala : Alanine
AMP : Adenosine monophosphate
Arg : Arginine
Arg Asn : Asparagine arginine
ARS : Autonomously replicating sequence
Asn : Asparagine
Asp : Aspartic acid
Asp : Aspartate
ATCase : Aspartate transcarbamoylase
atm : Atmosphere
ATP : Adenosine triphosphate
ATPase : Adenosine triphosphatase

BChl : Bacteriochlorophyll
bp : Base pair
BPG : D-2,3-bisphosphoglycerate
BPheo : Bacteriopheophytin
BPTI : Bovine pancreatic trypsin inhibitor

C : Cytosine
cal : Calorie
CaM : Calmodulin
cAMP : Cyclic 3',5'-adenosine monophosphate
cAMP : Cyclic AMP
cAMP : Adenosine 3',5'-cyclic monophosphate
CAP : Catabolite activating protein

CD : Circular dichroism
cDNA : Complementary DNA
CDP : Cytidine diphosphate
cGMP : Guanosine 3′,5′-cyclic monophosphate
cGMP : Cyclic GMP
Chl : Chlorophyll
CM : Carboxymethyl
CMP : Camp receptor protein (catabolite activator protein)
CMP : Cytidine monophosphate
CoA : Coenzyme A
CoA-SH : Reduced coenzyme A
CoQ : Coenzyme Q (ubiquinone)
cpm : Counts per minute
CTP : Cytidine triphosphate
Cys : Cysteine

d : Deoxy
Da : Dalton
dd : Dideoxy
DEAE : Diethylaminoethyl
DG : *Sn*-1,2-diacylglycerol
DHAP : Dihydroxyacetone phosphate
DHF : Dihydrofolate
DHFR : Dihydrofolate reductase
DMF : *N,N*-dimethylformamide
DMS : Dimethyl sulphate
DMSO : Dimethyl sulphoxide
DNA : Deoxyribonucleic acid
DNAse : Deoxyribonuclease
DNP : 2,4-dinitrophenol
Dol : Dolichol
dopa : L-3,4-dihydroxyphenylalanine
dTDP : Thymidine diphosphate
dTMP : Thymidine monophosphate
dTTP : Thymidine triphosphate

E : Reduction potential
EcoRI : *Eco*ri restriction endonuclease
EF : Elongation factor
EGF : Epidermal growth factor
EPR : Electron paramagnetic resonance
ER : Endoplasmic reticulum

F : Faraday constant

F1P : Fructose-1-phosphate
F6P : Fructose-6-phosphate
FAB : Antibody molecule fragment that binds antigen
FAD : Flavin adenine dinucleotide (oxidized form)
FAD : Flavin adenine dinucleotide
$FADH_2$: Flavin adenine dinucleotide (reduced form)
FBP : Fructose-1,6-bisphosphate
FBPase : Fructose bisphosphatase
Fd : Ferredoxin
FH : Familial hypercholesterolemia
fMet : *N*-formylmethionine
FMN : Flavin mononucleotide
FMN : Flavin mononucleotide (oxidized form)
$FMNH_2$: Flavin mononucleotide (reduced form)

G : Gibbs free energy
G : Guanine
G1P : Glucose-1-phosphate
G3P : Glyceraldehyde-3-phosphate
G6P : Glucose-6-phosphate
GABA : gamma-Aminobutyric acid
Gal : Galactose
GalNAc : *N*-acetylgalactosamine
GAP : Glyceraldehyde-3-phosphate
GAPDH : Glyceraldehyde-3-phosphate dehydrogenase
GDP : Guanosine diphosphate
Gla : γ-Carboxyglutamate
GLC : Gas-liquid chromatography
Glc : Glucose
Gln : Glutamine
Glu : Glutamic acid
Glu : Glutamate
Gly : Glycine
GMP : Guanosine monophosphate
GS : Glutamine synthetase
GSH : Glutathione (reduced glutathione)
GSSG : Glutathione disulphide (oxidized glutathione)
GTP : Guanosine triphosphate
GTPase : Guanosine triphosphatase

h : Hour
h : Planck's constant
HA : Haemagglutinin
Hb : Haemoglobin

HDL : High-density lipoprotein
HGPRT : Hypoxanthine-guanine phosphoribosyl transferase
His : Histidine
HIV : Human immunodeficiency virus
HMG-CoA : β-hydroxy- β -methylglutaryl-coa
hnRNA : Heterogeneous nuclear RNA
HPLC : High-pressure (or high-performance) liquid chromatography
HX : Hypoxanthine
Hyl : 5-hydroxylysine
Hyp : 4-hydroxyproline

IDL : Intermediate-density lipoprotein
IF : Initiation factor
IgG : Immunoglobulin 6
IHP : Inositol hexaphosphate
Ile : Isoleucine
IMP : Inosine monophosphate
InsP$_3$: Inositol 1,4,5-trisphosphate
IP$_1$: Inositol-1-phosphate
IP$_3$: Inositol 1,4,5-triphosphate
IP$_3$: Inositol 1,4,5,-trisphosphate
IPTG : Isopropylthiogalactoside
IR : Infrared
IS : Insertion sequence
ITP : Inosine triphosphate

J : Joule

k : Kilo (10^3)
kb : Kilobase
k_{cat} : Turnover number
kDa : Kilodalton
K_M : Michaelis constant

L : Liter
LCAT : Lecithin:cholesterol acyl transferase
LDH : Lactate dehydrogenase
LDL : Low-density lipoprotein
Leu : Leucine
Lys : Lysine

m : Milli (10^{-3})
M : Molar (mol/L)
Man : Mannose

Mb	:	Myoglobin
Met	:	Methionine
MHC	:	Major histocompatability complex
ml	:	Milliliter
mm	:	Millimeter
mM	:	Millimolar (mmol/L)
mmol	:	Millimole
mol	:	Mole
mRNA	:	Messenger RNA
mV	:	Millivolt

N	:	Avogadro's number
n	:	Nano (10^{-9})
NAD$^+$	:	Nicotinamide adenine dinucleotide (oxidized form)
NADH	:	Nicotinamide adenine dinucleotide (reduced form)
NADP$^+$	:	Nicotinamide adenine dinucleotide phosphate (oxidized form)
NADPH	:	Reduced nicotinamide adenine dinucleotide phosphate
NAG	:	*N*-acetylglucosamine
NAM	:	*N*-acetylmuramic acid
NANA	:	*N*-acetylneuraminic acid
nm	:	Nanometer
NMN	:	Nicotinamide mononudeotide
NMR	:	Nuclear magnetic resonance

p	:	Phosphate
p	:	Pico (10^{-12})
PAGE	:	Polyacrylamide gel electrophoresis
PBG	:	Porphobilinogen
PC	:	Phosphatidylcholine
PC	:	Plastocyanin
PCR	:	Polymerase chain reaction
PDGF	:	Platelet-derived growth factor
PE	:	Phosphatidylethanolamine
PEG	:	Pulsed-field gel electrophoresis
PEP	:	Phosphoenolpyruvate
PEPCK	:	PEP carboxykinase
PFK	:	Phosphofructokinase
PG	:	Prostaglandin
PGI	:	Phosphoglucose isomerase
PGK	:	Phosphoglycerate kinase
PGM	:	Phosphoglycerate mutase
Phe	:	Phenylalanine
Pheo	:	Pheophytin
P_i	:	Inorganic phosphate (also called orthophosphate ion)

PI : Phosphatidyl-inositol
PIP$_2$: Phosphatidylinositol-4,5-bisphosphate
PK : Pyruvate kinase
PKU : Phenylketonuria
PLP : Pyridoxal-5-phosphate
μm : Micrometer
Pol : Polymerase
PP$_i$: Pyrophosphate ion
PP$_i$: Inorganic pyrophosphate
PQ : Plastoquinone
Pro : Proline
PrP : Prion protein
PRPP : 5-phosphoribosyl-1-pyrophosphate
PS : Phosphatidylserine
PS : Photosystem
PSTV : Potato spindle tuber virus
PTH : Phenylthiohydantoin

Q : Ubiquinone (or plastoquinone)
QH$_2$: Reduced coenzyme Q (ubiquinol)

R : Gas constant
R5P : Ribose-5-phosphate
RER : Rough endoplasmic reticulum
RF : Release factor
RF : Replicative form
RFLP : Restriction fragment length polymorphism
RK : HMG-coa reductase kinase
RKK : Reductase kinase kinase
RNA : Ribonucleic acid
RNAse : Ribonuclease
rpm : Revolutions per minute
rRNA : Ribosomal RNA
RSV : Rous sarcoma virus
Ru1,5P : Ribulose-1,5-bisphosphate
Ru5P : Ribulose-5-phosphate
RuBP : Ribulose-1,5-bisphosphate

s : Second
S : Sedimentation coefficient
S : Svedberg unit
S7P : Sedoheptulose-7-phosphate
SAM : *S*-adenosylmethionine
scRNA : Small cytoplasmic RNA

SDS	Sodium dodecylsulfate
Ser	Serine
snRNA	Small nuclear RNA
snRNP	Small (nuclear) ribonucleoprotein
SRP	Signal recognition particle
T	Thymine
TBSV	Tomato bushy stunt virus
TCA	Tricarboxylic acid cycle
THF	Tetrahydrofolate
Thr	Threonine
TIM	Triose phosphate isomerase
TLC	Thin layer chromatography
TMV	Tobacco mosaic virus
TPP	Thiamine pyrophosphate
tRNA	Transfer RNA
Trp	Tryptophan
TTP	Thymidine triphosphate
Tyr	Tyrosine
U	Uracil
UDP	Uridine diphosphate
UDPG	UDP-glucose
UDP-galactose	Uridine diphosphate galactose
UDP-glucose	Uridine diphosphate glucose
UMP	Uridine monophosphate
UTP	Uridine triphosphate
UV	Ultraviolet
Val	Valine
VLDL	Very low-density lipoprotein
V_{max}	Maximal velocity
XMP	Xanthosine monophosphate
Xu5P	Xylulose-5-phosphate
YAC	Yeast artificial chromosome
YADH	Yeast alcohol dehydrogenase
µM	Micromolar (µmol/L)
µmol	Micromole
µ	Micro (10^{-6})
2PGA	2-phosphoglycerate
3PGA	3-phosphoglycerate

“This Page is Intentionally Left Blank”

Index

Alanine Cycle, 437
Albuminoids, 150
Albumins, 149
Aldolase, 282
Aldonic Acids, 53
Aldoses, 48
Allosterism, 166
Amino Acid Oxidase, 433
Amino Acids, 139, 294
Amino Sugars, 52
Amino transferases, 431
Amylopectin, 58
Amylose, 58
Anabolism, 257
Antibiotics, 450
Apoenzymes, 184
ATP Synthase, 315, 478
Autotrophs, 475

Benzoic Acid, 437
Bilayer Formation, 92
Biochemistry, 20
Biological Membranes, 91
Biomolecules to Cells, 5
Biotin, 230
Branched Chain Amino Acids, 439
Brassinosteroids, 88
Buffers, 19

C4 Metabolism, 484
CAM Metabolism, 489
Capping, 394
Capsule, 8
Carbohydrates, 44, 262
Carnitine Carrier, 347
Catabolism, 257
Cell Envelope, 9
Cell Wall, 9
Cells, 7
Cellulose, 61
Chitin, 63
Chromoproteins, 150
Chymotrypsin, 429
Citrate Synthase, 299
Coagulated Proteins, 151
Cobalamin, 231
Compound Lipids, 82
Contractile Proteins, 153
Cytoplasm, 9
Cytoplasmic Membrane, 9

Dark Reactions, 472
Decarboxylation, 433
Deoxy Sugars, 52
Deoxycoformycin, 385
Deoxyribonucleotide Synthesis, 384
Derived Lipids, 78
Derived Proteins, 151
Derived Sugars, 52
Disaccharides, 55
DNA, 117
DNA Polymerase, 401

Elemental Ions, 4
Elements of Life, 3
Endopeptidases, 428
Enolase, 287
Enzyme Regulation, 196
Enzymes, 181
Essential Amino Acids, 146
Essential Fatty Acids, 80
Eukaryote Cell, 11
Exopeptidases, 428

Fat System, 343
Fat-Soluble Vitamins, 225
Fatty Acid Biosynthesis, 357
Fatty Acids, 78, 345
Flagella, 10
Fluid Mosaic Model, 93

Folic Acid, 231
Fuel, 355

Genetic Code, 443
Globulins, 149
Gluconeogenesis, 292
Glutamine, 439
Glutelins, 149
Glycerol, 295
Glycerophospholipids, 82
Glycogen Metabolism, 303
Glycogenesis, 303
Glycogenolysis, 306
Glycolipids, 85, 94, 95
Glycolysis, 276
Gout, 382
G-Proteins, 97
Gums, 66

Haemoglobin, 165
Hemicelluloses, 63
Histidine, 439
Histones, 149

Inhibitors, 193
Inulin, 60
Irreversible Inhibitors, 193
Isoenzymes, 196

Ketogenesis, 352
Ketone Bodies Metabolism, 351
Ketoses, 49
Koshland's Induced-fit Theory, 186
Kreb's Tca Cycle, 297
Kreb's Urea Cycle, 433

Lactate, 294
Lesch-Nyhan Syndrome, 382
Light Reactions, 472
Lipids, 77
Lipogenesis, 362
Lipoic Acid, 231
Lipolysis, 345
Lipoprotein, 86
Lipoproteins, 150

Membrane Carbohydrates, 93
Metabolic Pathways, 257
Metalloproteins, 150
Metaproteins, 151
Methotrexate, 385
Mitochondrial Transport, 316
Monosaccharides, 44
Mucoproteins, 150
Myoglobin, 162

Niacin, 229
Nitrogen Economy, 451
Nomenclature, 43
Nucleic Acid Building Blocks, 113
Nucleoid, 10
Nucleoproteins, 150
Nucleoside, 114

Odd Chain Fatty Acids, 350
Oligosaccharides, 44, 54
Optical Specificity, 182
Organelles, 7
Origin of Life, 1
Ornithine, 439
Oxidative Phosphorylation, 315

Pantothenic Acid, 230
Papain, 429
Pectins, 63
Peptidases, 428
Peptides, 148
Phosphatidyl choline, 82
Phosphatidyl ethanolamine, 83
Phosphatidyl glycerol, 84
Phosphofructokinase Regulation, 281
Phosphoglycerate Kinase, 286
Phosphoglycerate Mutase, 286
Phosphoproteins, 151
Photorespiration, 484
Photosynthesis, 471
Pili, 10
Plant Cell, 16

Plasmids, 10
Plastoquinone, 476
polar amino acids, 141
Polyols, 52
Polysaccharides, 44, 58
Prokaryote Cell, 7
Proline, 439
Promoters, 391
Protamines, 149
Proteans, 151
Protein Biosynthesis, 448
Protein Catabolism, 428
Protein Maturation, 449
Proteins, 262
Purine Metabolism, 379
Purines, 113
Pyridoxine, 229
Pyrimidines, 113
Pyruvate Kinase, 288

Reversible Inhibitors, 194
Riboflavin, 228
Ribosome, 446
Ribosomes, 11
RNA, 119
RNA Synthesis, 386

Saccharic Acid, 54
Secondary-derived Proteins, 151
Simple Lipids, 80
Sphingolipids, 94
Structural Polysaccharides, 61
Sulpholipids, 85

Telomerase, 407
Thiamine, 227
Toxic Proteins, 153
Toxins, 451
Transport Proteins, 152
Trypsin, 429
Turnover Number, 191

Urea Cycle, 433
Uronic Acids, 53

Vitamins, 264

Water, 16
Water-Soluble Vitamins, 226